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# CANCER OF THE UTERINE CERVIX AND SOCIAL CONDITIONS

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*Marriage, infertility and social class*

The Registrar-General of England and Wales commenced in 1950 to include amongst the subjects for medical inquiry concerning statements on death certificates cancer of the " uterus " without specification of the part of the uterus in which the neoplasm was present or was thought to have originated. The results of this have been such as to produce meaningful statistics of cervix cancer mortality which were never obtainable up to that time. In order to assist my work for the British Empire Cancer Campaign the General Registrar Office kindly extracted for me the numbers of deaths attributed to this cause during 1950 and 1951, classified according to age, marital condition and, in the case of married women, the social class of the husband and whether or not there had been any children. From these data in respect of 5287 deaths the rates and comparative mortality figures shown in Table I were computed by relating the deaths, after correction

TABLE I.—*Death-rates per Million from Cancer of Cervix Uteri, by Age and Marital Condition, and by Fertility and Social Class of Married Women. Comparative Mortality Ratios in terms of All Married Women taken as* 100. *England and Wales,* 1950–51.

| | Age group. | Single all. | Married. Infertile. | Married. All. | Married. Fertile. | Widowed and divorced (all). |
|---|---|---|---|---|---|---|
| Death rates | 25– | 9 | — | 20 | — | 41 |
| | 35– | 39 | — | 70 | — | 205 |
| | 45– | 69 | 188 | 191 | 192 | 295 |
| | 55– | 136 | 244 | 314 | 325 | 395 |
| | 65 and over | 150 | 306 | 346 | 352 | 423 |
| C.M.R. | All ages | 43 | 84 | 100 | 103 | 125 |

*Married Women by Social Class.*

| | Age group. | I. | II. | III. | IV. | V. |
|---|---|---|---|---|---|---|
| Death rates | 25– | 16 | 14 | 19 | 23 | 36 |
| | 35– | 61 | 50 | 75 | 73 | 103 |
| | 45– | 140 | 131 | 194 | 205 | 255 |
| | 55– | 203 | 234 | 310 | 323 | 430 |
| | 65 and over | 310 | 327 | 348 | 345 | 376 |
| C.M.R. | All ages | 76 | 77 | 101 | 104 | 131 |

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for those not classifiable as to social class or fertility, to the corresponding population derived from the Census of 1951 (Registrar-General, 1953).

There are four variables in the table—age at death, civil state at death, social class of husband and whether there had been a live born child in the case of married women. The social class is based on the occupation of the husband at the time of the wife's death ; Group V comprising unskilled occupations, IV partly skilled, III skilled, II an intermediate group mainly managerial, and I consisting of professional and high administrative occupations. The populations calculated from the sample taken from the census are subject to sampling errors and the final rates when they become available will differ a little from the estimates in Table I, but not sufficiently to affect the conclusions to be drawn in this paper.

Infertile married women suffered about double the mortality of single women at corresponding ages ; and the C.M.R. was about 23 per cent higher for fertile than for infertile married women, the difference not appearing until after age 55. In Social Class V mortality was about 30 per cent greater than in Classes III and IV, which in turn showed an excess of about 35 per cent over Classes I and II, but at ages over 65 the class differences were relatively much slighter than at earlier ages.

As shown below, the relations with civil state, fertility and social class are very different from those given by breast cancer in the same years ; there is for the latter no difference between single and infertile married women, who in their turn suffer a mortality 1½ times that of fertile married women, and when the social classes are compared the C.M.R. is highest in Class I and lowest in Class V.

| | C.M.R. for cervix. | C.M.R. for breast. |
|---|---|---|
| Single women | 43 | 136 |
| Married—infertile | 84 | 139 |
| fertile | 103 | 93 |
| Widowed and divorced | 125 | 121 |
| Married—Class I | 76 | 133 |
| Class V | 131 | 91 |

The contrast between cervix and breast in their relations with social class amongst married women was anticipated from the findings for uterus as a whole in the occupational analysis of deaths in 1930–32 (Registrar-General, 1938), and Logan (1954) gave some preliminary figures for cervix cancer deaths in 1950 which showed the same contrast. The purpose of this paper is to examine some social factors which might be responsible for the class gradient shown by the cervix cancer death-rates at ages before 65. These might be higher fertility and earlier marriage in Class V, overcrowding with consequent effects on hygiene, employment of women or of men in particular kinds of occupation.

*Age at marriage and number of confinements*

The fact of having had at least one child has only a small association with death-rates from cervix cancer, as Table I showed, and the relationship could be secondary to a strong association with early marriage. It is possible to ascertain from the data what would be the effect of correcting the social class rates for differing degrees of infertillity in the classes, since age-standardised rates of infertility can be calculated for married women of each class. Expressing these in terms of the figure for Class III, the relative infertility rates were 1·06, 1·02, 1·00,

0·84 and 0·87 in Classes I, II, III, IV and V respectively, and from these the percentages expected to be infertile amongst the women who died of cervix cancer can be found and corrections applied to the death rates in Table I. The effect on the social class gradient is very slight, the corrected comparative ratios being as follows :

| Age group. | I. | II. | III. | IV. | V. |
|---|---|---|---|---|---|
| 45– | 73 | 69 | 102 | 107 | 134 |
| 55– | 65 | 75 | 99 | 103 | 137 |
| 65+ | 90 | 95 | 101 | 99 | 108 |

Comparisons between women with cervix cancer and control groups in respect of the numbers of children they have had show almost invariably a larger average family size for the cervix group, but it is necessary to find out whether this is true amongst women of completed fertility who had married at the same age, and conversely whether amongst women of equal completed fertility cervix cancer occurs more often in those who had married young or who had been in the married state for a long period of years. Data for such a study are being collected in the course of the 5-year survey of all forms of cancer by the Cheshire and North Wales Branch of the British Empire Cancer Campaign, and the first 155 cases of cervix cancer recorded at ages 45–74 in Merseyside have been classified in Table II according to present age, age at first marriage, duration of married partnership

TABLE II.—*Distribution of* 155 *Women with Cancer of the Cervix Uteri at Ages* 45–74 *in Merseyside, according to Age at Marriage, Number of Years with Husband and Number of Confinements, compared with expected frequencies derived from histories of* 718 *Hospital patients without cancer.*

| Age at marriage. | Number of women classified by years of married partnership and present age. | | | | | | Total women of present ages 45–74. | |
|---|---|---|---|---|---|---|---|---|
| | Less than 25 years. | | | 25 years or more. | | | | |
| | 45– | 55– | 65–74 | 45– | 55– | 65–74 | Actual. | Expected.* |
| 16–19 | 5 | 4 | 4 | 12 | 6 | 5 | 36 | 20 |
| 20–24 | 7 | 2 | 3 | 13 | 26 | 13 | 64 | 68 |
| 25–34 | 7 | 4 | 1 | 2 | 17 | 11 | 42 | 52 |
| 35+ | 2 | 2 | 3 | — | 1 | — | 8 | 10 |
| Totals: | | | | | | | | |
| Married | 21 | 12 | 11 | 27 | 50 | 29 | 150 | 150 |
| Single | 5 | — | — | — | — | — | 5 | 15 |

| | Number of women whose confinements numbered : | | | Total numbers of confinements : | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Of women now aged : | | | Of all women : | |
| | 0, 1, 2 | 3, 4 | 5 or more | 45– | 55– | 65–74 | Actual. | Expected.† |
| 16–19 | 8 | 4 | 24 | 79 | 60 | 51 | 190 | 199 |
| 20–24 | 17 | 26 | 21 | 82 | 103 | 88 | 273 | 288 |
| 25–34 | 18 | 15 | 9 | 29 | 67 | 36 | 132 | 99 |
| 35+ | 7 | 1 | — | 0 | 2 | 5 | 7 | 3 |
| Total | 50 | 46 | 54 | 190 | 232 | 180 | 602 | 589 |

* Number expected if the ages at marriage within each age group had been distributed as in the controls.

† Numbers expected, standardised for age of patient, age at marriage and years with husband by applying the rates in corresponding sub-groups of the control series.

and number of confinements. The expected distribution of women resident in the same area according to age at marriage, and the expected numbers of confinements, are calculated from the histories of 718 patients who were admitted to Broadgreen Hospital, Liverpool, without a diagnosis of any form of cancer, this control group being properly weighted according to present age.

After correction for all the variables in this way, the cervix group shows a pronounced excess of women who had married before age 20, the actual number being 36 compared with 20 expected in such a population, with corresponding deficiencies in the age groups after 25. The number of single women was only 5 compared with 15 expected. The numbers of confinements show no excess over expectation amongst those who had married before 25, but considerable excess amongst those who had married after that age. This suggests that multiplicity of confinements does not add to the risk of cervix cancer when marriage occurs early, but if marriage is delayed, many deliveries or the marital and social habits responsible for them, may increase the risk.

Although the numbers of cases available as yet are rather small, the interim analysis in Table II is being reported at this stage because it points to the same conclusion as that reached in a recent carefully planned investigation of patients in nine hospitals in the United States of America (Wynder *et al.*, 1954). In that study histories were obtained from 354 white non-Jewish women, 20 Jewish, 215 Negro, 255 Hindu and 48 other Indian women, and from 1526 control patients in the same hospitals. In the white non-Jewish group 2 per cent had never married, compared with 9 per cent of control cases at the same ages (cp. 3 and 9 per cent in Table II); 14 per cent had married before 16, compared with 8 per cent of the controls, and 19 per cent stated that first intercourse had occurred before 16, compared with 10 per cent of the controls (cp. 23 per cent married before 20 against 12 per cent expected, in Table II). Comparing the numbers of pregnancies in Wynder's group of 346 married white non-Jewish women and 205 Negro women with those expected from the controls, the following result appears :

| | White non-Jewish. | | | | Negro women. | | | |
|---|---|---|---|---|---|---|---|---|
| | By number of pregnancies. | | Per cent with 5 or more. | | By number of pregnancies. | | Per cent with 5 or more. | |
| Age at marriage. | 0–4 | 5+ | Actual. | Expected. | 0–4 | 5+ | Actual. | Expected. |
| Under 20 | 109 | 58 | 42 | 41 | 97 | 43 | 31 | 31 |
| 20–24 | 74 | 27 | 27 | 21 | 33 | 10 | 23 | 24 |
| 25 and over | 50 | 8 | 14 | 8 | 22 | 0 | 0 | 4 |

No association with high fertility is present in either group when marriage had occurred before 20, but amongst the white women married after 20 such an association does appear, just as it does in Table II for Merseyside women who married after 25. No connection was found by Wynder between cervix cancer incidence and ages at first or last pregnancies amongst women who married at the same age.

In view of the agreement between these studies, and taking account also of the rarity of cervix cancer amongst virgins who have passed middle age (Gagnon, 1950) and the low rates amongst single women generally, there can be no doubt that sex intercourse starting at an early age and continuing over many years is an important factor in causation, except in certain circumstances. This need not be the case if marital hygiene is meticulously observed, for example morbidity surveys by the National Cancer Institute as reported by Dorn (1955) showed an incidence amongst

Jewish women in Israel which is about one sixth of that amongst white women in the U.S.A. There is plenty of evidence accumulating on the question of marital habits practised by certain racial and religious groups, but this paper is not concerned with these, but with possible effects of environment in increasing the risk of cervix cancer in populations not adopting special measures to protect them. It seems fair to conclude from what is now known that in such populations the risk is a function of the total amount of sex intercourse, and that confinements directly contribute little or nothing to the risk when marriage occurs early. The association with size of family when marriage is delayed may well arise from association between family size and environmental conditions which themselves directly affect the risk of cervix cancer and are at the same time accompanied by a tendency to larger families. Some of these environmental conditions are examined in the next section.

*Industrial and social conditions in large towns*

In the three years 1950–52 there were 2257 deaths attributed to cancer of the cervix amongst residents in the 48 County Boroughs of England and Wales having more than 100,000 population. By applying the death-rates in all county boroughs at different ages to the census populations and expressing the actual deaths at all ages in terms of the calculated totals, taken as 100 in each instance, comparative mortality ratios are obtained which are free from disturbances caused by differing age-distributions in the populations. Table III gives the numbers of deaths and the C.M.R.'s, and the 48 towns have been ranked in descending order of the latter, ranging from 197 for South Shields to 63 for Bournemouth. In the next two columns are two indices of social conditions 20 years before, namely the crowding index of persons per room and the proportion per 1000 of men aged 14 and over who were in Social Classes IV and V, these indices in 1931 being taken from Table XCVII of the Registrar-General's *Statistical Review* for 1934, text. In the succeeding columns the towns are classified into 5 industrial groups ; (*a*) seaports ; (*b*) other seaboard towns which are residential or holiday resorts ; (*c*) inland towns having 10 per cent or more of men over 14 in the textile industry in 1931 and consequently employing many women also in textile work ; (*d*) inland manufacturing towns with 30 per cent or more of men over 14 in mining, pottery, metal, chemical, leather and textile industries but not qualifying for inclusion in Group (*c*) ; (*e*) other inland towns. Data for the classification of the towns by industry were derived from Table XCVII of the *Statistical Review* for 1936, text.

Dividing the series of 48 towns into 4 groups of 12, as in Table III, the relationship between the C.M.R. for cervix cancer and the various characteristics of the towns can be seen in Table IV.

The towns with high cervix mortality tend to have had greater densities of persons per room and larger proportions of men in Social Classes IV and V. Amongst the 30 inland towns 7 were predominantly textile (*c*), and of these 6 gave C.M.R.'s exceeding 112 ; 8 others had a large proportion of heavy manufacture (*d*) and of these only two gave high mortality ratios, whilst the remaining 15 inland towns included only 3 with a high C.M.R. From this it would appear that in textile towns, where many women also work in textile mills, there is some special factor increasing the risk of cervix cancer. It may be recalled that in his work on skin cancers notified under the Factory Acts in the period 1920–49, Henry (1950) found

TABLE III.—*Comparative Mortality (Standardised) from Cancer of the Cervix Uteri in* 1950–52 *in County Boroughs of England and Wales with over* 100,000 *Population, and Indices of Social and Industrial Conditions in* 1931.

| | Cervix cancer. | | | Industrial grouping. | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Seaboard. | | Inland towns.† | |
| | Deaths 1950–52. | C.M.R. | Persons per room. | Social class index.* | Ports. | Other. | (*c*). | (*d*). | (*e*). |
| South Shields | 34 | 197 | 1·18 | 431 | + | — | — | — | — |
| Middlesbrough | 39 | 183 | 1·03 | 493 | + | — | — | — | — |
| Bradford | 101 | 175 | 0·88 | 361 | — | — | + | — | — |
| Gateshead | 31 | 175 | 1·23 | 431 | + | — | — | — | — |
| Kingston-on-Hull | 81 | 172 | 0·89 | 470 | + | — | — | — | — |
| Southampton | 49 | 163 | 0·80 | 339 | + | — | — | — | — |
| York | 30 | 162 | 0·84 | 394 | — | — | — | — | + |
| Newcastle-upon-Tyne | 75 | 152 | 1·13 | 386 | + | — | — | — | — |
| Newport | 26 | 152 | 0·87 | 413 | + | — | — | — | — |
| Nottingham | 77 | 147 | 0·75 | 359 | — | — | — | — | + |
| Stoke-on-Trent | 62 | 145 | 1·04 | 404 | — | — | — | + | — |
| Leeds | 129 | 141 | 0·87 | 333 | — | — | — | — | + |
| Oldham | 31 | 135 | 0·89 | 376 | — | — | + | — | — |
| Preston | 29 | 134 | 0·85 | 373 | — | — | + | — | — |
| Bolton | 42 | 131 | 0·85 | 360 | — | — | + | — | — |
| Cardiff | 53 | 129 | 0·86 | 358 | + | — | — | — | — |
| Plymouth | 45 | 129 | 0·96 | 283 | + | — | — | — | — |
| Portsmouth | 51 | 127 | 0·76 | 260 | + | — | — | — | — |
| Blackpool | 45 | 126 | 0·67 | 259 | — | + | — | — | — |
| Liverpool | 149 | 119 | 0·93 | 434 | + | — | — | — | — |
| Stockport | 32 | 118 | 0·82 | 343 | — | — | + | — | — |
| St. Helens | 19 | 117 | 1·13 | 480 | — | — | — | + | — |
| Huddersfield | 29 | 114 | 0·91 | 343 | — | — | + | — | — |
| Swansea | 29 | 113 | 0·90 | 390 | + | — | — | — | — |
| Coventry | 42 | 112 | 0·85 | 342 | — | — | — | + | — |
| Blackburn | 26 | 111 | 0·82 | 322 | — | — | + | — | — |
| Manchester | 135 | 110 | 0·87 | 352 | — | — | — | — | + |
| Sheffield | 97 | 109 | 0·87 | 382 | — | — | — | + | — |
| Birkenhead | 25 | 106 | 0·89 | 418 | + | — | — | — | — |
| East Ham | 22 | 106 | 0·93 | 311 | — | — | — | — | + |
| Derby | 25 | 102 | 0·75 | 326 | — | — | — | + | — |
| Norwich | 23 | 100 | 0·71 | 303 | — | — | — | — | + |
| Wallasey | 20 | 99 | 0·71 | 241 | — | + | — | — | — |
| Leicester | 55 | 98 | 0·69 | 291 | — | — | — | — | + |
| Ipswich | 18 | 97 | 0·69 | 331 | — | — | — | — | + |
| Salford | 29 | 97 | 0·94 | 424 | — | — | — | — | + |
| West Ham | 25 | 95 | 1·14 | 483 | — | — | — | — | + |
| Brighton | 32 | 89 | 0·79 | 387 | — | + | — | — | — |
| Bristol | 71 | 87 | 0·81 | 350 | — | — | — | — | + |
| Birmingham | 156 | 86 | 0·83 | 323 | — | — | — | + | — |
| Oxford | 15 | 82 | 0·72 | 268 | — | — | — | — | + |
| Croydon | 39 | 80 | 0·75 | 211 | — | — | — | — | + |
| Reading | 17 | 80 | 0·75 | 330 | — | — | — | — | + |
| Wolverhampton | 20 | 77 | 0·87 | 350 | — | — | — | + | — |
| Southend-on-Sea | 25 | 74 | 0·79 | 196 | — | + | — | — | — |
| Northampton | 15 | 74 | 0·70 | 256 | — | — | — | — | + |
| Walsall | 12 | 68 | 0·91 | 375 | — | — | — | + | — |
| Bournemouth | 25 | 63 | 0·64 | 199 | — | + | — | — | — |

* Men in Classes IV and V per 1000 in all classes.

† Subgroup (*c*) had over 10 per cent of men in textile work; (*d*) had over 30 per cent in mining, pottery, metal, chemical, leather and textile industries, but not as in (*c*); (*e*) are the remaining inland towns with less heavy industry.

TABLE IV.—*Summarised View of the Distribution in Table III.*

| Range of C.M.R. | Number of towns. | Mean per room index. | Mean social index. | Industrial grouping of towns: Seaboard. Ports. | Seaboard. Other. | Inland. (c). | Inland. (d). | Inland. (e). |
|---|---|---|---|---|---|---|---|---|
| 63– 95 | 12 | 0·808 | 311 | — | 3 | — | 3 | 6 |
| 97–112 | 12 | 0·810 | 337 | 1 | 1 | 1 | 3 | 6 |
| 113–135 | 12 | 0·877 | 355 | 5 | 1 | 5 | 1 | — |
| 141–197 | 12 | 0·962 | 401 | 7 | — | 1 | 1 | 3 |
| All | 48 | 0·867 | 351 | 13 | 5 | 7 | 8 | 15 |

that out of 3530 persons affected 1490 worked in textile occupations, and of these 1349 were mule spinners; and from analysis of 25,645 deaths with mention of skin cancer on the death certificate during the period 1911–45 he found high rates amongst textile workers.

Even more remarkable is the distribution of seaboard towns, since out of 13 seaports no less than 12 give mortality ratios exceeding 113, whereas this was true of only 1 out of 5 other seaboard towns. Either the social conditions characterising seaports or some specific factor arising out of mercantile occupations of men, or both of these, increase the risk of cervix cancer in the female populations. It may be relevant in this connection that in his analysis of 1638 deaths from cancer of the scrotum during 1911–38 Henry (1946) found high rates amongst fishermen and workers with tar and pitch, and in the larger series of deaths with mention of any form of skin cancer fishermen were again amongst the occupations showing high rates.

In a previous paper (Stocks, 1953) it was calculated from the statistical association between cervix cancer incidence and family size that the progressive fall in the birth rate during the first few decades of this century should have diminished the death rates in the course of the last 20 years by about 8 per cent, whereas actually a greater fall than that seems to have occurred. Although the reasons for the connection with number of confinements would appear to be more indirect than direct, the factors now thought to be directly operative were also so changing during the period as to lead to an expectation of declining risk of cervix cancer in later life, and the general conclusions from the 1953 study need little modification.

## SUMMARY

Death-rates from cervix cancer in England and Wales in 1950–51 were much higher for childless married women than for single women, and rates at ages under 65 were particularly high amongst wives of unskilled labourers. A preliminary comparison of the histories of 155 women aged 45–74 with cervix cancer in Merseyside with those of a control group of 718 in a Liverpool hospital indicates that early marriage is a more important factor than number of confinements, which agrees with Wynder's finding from a similar study of white women in American hospitals (Wynder *et al.*, 1954).

Within groups of women who married at the same ages after 25 the association between cervix cancer incidence and completed family size probably results indirectly from environmental conditions which are directly conducive to the disease and also often associated with high fertility. A study of the death-rates in

48 large towns in 1950–52 shows a relationship with overcrowding, social class distribution and predominant industry 20 years before. Seaports and textile towns have high rates for cervix cancer, and possible reasons for this are discussed.

Thanks are due to the Medical Officers and Health Visitors of the Merseyside boroughs and Medical Staff of the Radium Institute for their help in obtaining the histories of the cancer patients, to the Management Committee of Broadgreen Hospital for their co-operation in respect of the histories both of cancer and control patients, and to the General Register Office for supplying details of deaths according to social class and fertility.

## REFERENCES

DORN, H. F.—(1955) *Publ. Hlth. Rep., Wash.*, **70**, 219.

GAGNON, F.—(1950) *Amer. J. Obstet. Gynec.*, **60**, 516.

HENRY, S. A.—(1946) 'Cancer of the Scrotum in Relation to Occupation.' London (Oxford University Press).—(1950) *Ann. R. Coll. Surg. Engl.*, **7**, 425.

LOGAN, W. P. D.—(1954) *Brit. J. preventive soc. Med.*, **8**, 128.

REGISTRAR-GENERAL.—(1938) Decennial Supplement for 1931. 'Occupational Mortality.' London (H.M. Stationery Office).—(1953) 'Census of 1951 One per cent Sample Tables,' Part 2. London (H.M. Stationery Office).

STOCKS, P.—(1953) *Brit. J. Cancer*, **7**, 283.

WYNDER, E. L., CORNFIELD, J., SCHROFF, P. D. AND DORAISWAMI, K. R.—(1954) *Amer. J. Obstet. Gynec.*, **68**, 1016.

# LUNG CANCER AND TOBACCO SMOKING IN NORWAY

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In a series of papers the lung cancer occurrence in Norway has been analysed. The study has been based upon a careful histological typing and subsequent grouping of the material.

All cases of primary epithelial lung tumours have been regarded as "lung cancer", including adenomas and salivary gland tumours. The latter two types are qualified for inclusion partly by the fact that diagnostically, in most cases, the patients present the same set of clinical symptoms as the more malignant tumours, and more especially because they are included in the usual mortality statistics in most countries (Kreyberg, 1954*a*). Many of the adenomas and salivary gland tumours are fatal tumours, in spite of certain formal features qualifying the designations benign or semimalignant.

To avoid secondary lung tumours being included, a very careful scrutiny of the case histories has been made in each case of the present material. Metastases from breast carcinomas and cervix carcinomas in females may, in the lung, present pictures very like poorly differentiated squamous cell carcinomas. Metastases from adenocarcinomas of the ovary, the uterus and the stomach may likewise lead to diagnostic difficulties, as regards primary adenocarcinomas, or bronchiolar cell carcinomas.

Accordingly, in this material only such cases have been included which were found, beyond reasonable doubt, to be primary epithelial lung tumours, and in which the histological material was sufficient in quality and in quantity to permit a definite histological typing.

The histological typing has resulted in a subdivision of the primary epithelial lung tumours as follows; Squamous cell carcinomas, large cell carcinomas, small cell ("oat-cell") carcinomas, adenocarcinomas, bronchiolar cell ("alveolar" cell) carcinomas, adenomas ("benign", "semimalignant" and "malignant"), salivary gland tumours, comprising cylindromas and more or less mucus producing tumours with a salivary gland pattern. For the criteria and the qualification of the establishment of these types, the reader may consult the papers of Kreyberg (1952 and 1954*a*). In the latter paper it has been shown that the histologically different types of primary epithelial lung tumours show special biological properties, indicating differences in etiology and development.

Squamous cell, large cell and small cell carcinomas show an identical age-group incidence, and a striking sex difference with a great preponderance of male victims. They seem to be histological variants of an oncological entity. These are the tumours which occur in relation to special carcinogenic agents, such as were seen, for instance, in the miners in Schneeberg and Joachimsthal, and which occur in certain workers in the nickel industry. These tumours have been designated Group I tumours by the present author.

The remaining primary epithelial lung tumours form a more heterogeneous group. The adenomas and salivary gland tumours, by their equal sex distribution and their occurrence in all age-groups, including infancy, are most probably of developmental origin. The bronchiolar cell carcinomas present similar characteristics; they may be of developmental origin, or they may be caused by a virus infection. The adenocarcinomas, finally, show an equal sex distribution, but a steadily increasing occurrence with advancing years, pointing to etiological factors hitting males and females with equal force. The tumours mentioned in this paragraph have, in spite of certain differences, been grouped together, forming the author's Group II tumours.

On the basis of this grouping it has been shown that a numerical increase in lung cancer has been registered in Norway, in both sexes, as a result of better diagnostic facilities, and that a definite real increase in the incidence of lung tumour in males has taken place since the middle of the nineteen forties, an increase caused solely by Group I tumours (Kreyberg, 1954*b*). This increase in the Group I tumours in males is, as yet, limited to the urban population, and with the most marked rise in the capital. The rural population does not yet show such an increased occurrence, nor has the ratio of the histological types changed in rural areas (Kreyberg, 1954*c*).

It has been shown furthermore that no special occupation in Norway gives rise to a definitely increased lung cancer occurrence, although work with metal dust and metal fumes may be of significance. Domestic workers and open air workers show, as occupational categories, the lowest figures, individuals with dusty work show the highest, and clerical and professional workers range in between. The latter category, however, is closer to the workers in dusty industries and trades, than to the open air workers (Kreyberg, 1954*d*).

The general conclusions of these studies were (i) that the subdivision of the lung cancer cases into the two main groups has been shown to be a fruitful technical method of investigation, (ii) that the Group I tumours have increased in number in recent years in males living in urban districts, (iii) that this increase is caused by a new carcinogenic situation, and (iv) that this new situation probably consists more in changes in the male's life habits than in his working conditions.

In the present paper the same methodology will be used in the study of the relationship between smoking habits and lung cancer.

In the literature it has previously been stated that: "The influence of tobacco on the development of adenocarcinoma seems much less than on the other types of bronchiogenic carcinoma" (Wynder and Graham, 1950).

Lickint (1953), in discussing etiological factors in lung cancer, divides the mode of lung cancer occurrence into three groups; Group A comprises the female lung cancers in the first decades of this century. Group B comprises the male lung cancer occurrence at the beginning of this century, with slightly higher figures than for the females, especially as regards squamous cell carcinomas ("Plattenepithel Karzinome"), and finally Group C covering the enormous rise in male lung cancer in the last few decades, caused mainly by squamous cell carcinomas. Lickint designates tumours of Group A as the gene-linked ("gen-gebundene") tumours, and these he enumerates as; Adeno- and bronchiolar cell carcinomas, and some squamous cell carcinomas. Lickint does not draw any principal and formal line between these types, but builds his grouping upon the actual numerical occurrence of such types, including some of the squamous cell carcinomas in the gene-linked tumours. Breslow, Hoaglin, Rasmussen and Abrams,

(1954) state that " Some support for the hypothesis that cigarette smoking affects the development of epithelial carcinoma of the lung more than adenocarcinoma may be noted."

Finally Wynder (1954), in a recent publication has investigated the smoking habits of 1019 cases of " epidermoid " carcinomas, including " oat-cell " carcinomas and 85 cases of adenocarcinoma, including bronchiolar cell carcinomas. In the first groups he found 1·4 per cent non-smokers in males, in the later 10 per cent. non-smokers in males. The respective figures for the females were 40 per cent and 84 per cent. Wynder finds that the number of non-smokers in the general hospital population is very close to that of the " glandular type tumour " patients

The present study is based upon 300 cases, 258 males and 42 females. The number of the different histological types will be seen from Table I.

TABLE I.—*Total Material.*

| | | Males. | Females. |
|---|---|---|---|
| Group I tumours | Squamous cell carcinomas | 147 | 2 |
| | Large cell carcinomas | 12 | 0 |
| | Small cell carcinomas | 54 | 3 |
| Total | | 213 | 5 |
| Group II tumours | Adenocarcinomas | 19 | 17 |
| | Bronchiolar cell carcinomas | 7 | 5 |
| | Adenomas | 13 | 12 |
| | Salivary gland tumours | 6 | 3 |
| Total | | 45 | 37 |

In all cases a questionaire has been completed, giving present age, age when smoking was commenced and if and when ended, the amount smoked, given as number of cigarettes, or as number of cigars per day, or as grams smoked per week in the case of pipe smoking and hand-rolling of cigarettes. One cigarette is counted as 1 gram.

The first part of the analysis will be a comparison of the smoking habits of the lung tumour patients with the " control " material presented by Kreyberg (1954) in his study of smoking habits in Norway. The weakness of any type of control material has been discussed in that paper and will not be further considered here. The original " control " material consisted of 7 male and 4 female groups. These have now been reduced, for the following reasons. From both the males and from the females, the patients from the Ear, Nose and Throat Department have been omitted. These two groups are small and they represent a special group of individuals with a possible positive or negative relationship to smoking. Furthermore, from the male " control " material the Kongsberg workers have been omitted. They showed a sufficiently lower smoking level than the other " control " groups to cause comment in the publication of Kreyberg (1954). After that publication, the local factory physician wrote and gave me the information that he, for years, had systematically advised the workers against smoking. The remaining " control" material consists of 4172 males and 997 females.

First, the lung tumour material was typed and subsequently placed in our Group I or Group II. Thereafter, each tumour group has been arranged according to the corresponding age-groups and the same groups of smoking levels as used in

the " control " study. Next, a corresponding number of cases from each age-group of the " control " material have been taken, and tabulated according to the occurrence of smoking levels of that " control " material, age-group for age-group. Finally, the percentage figures for smoking in each age-group of the " control " material were multiplied by the number of cases of lung tumour observed in the same age-group, and the products were added for all ages. The numbers obtained in this way, for each level of smoking, were then compared with the numbers actually observed in the series of lung tumour cases. The basic figures for the " control " material are given in Appendix, Table I.

In our previous papers it has been shown that the Group II tumours occur with practically equal frequency in the two sexes, and that they are evenly distributed over the whole country of Norway, without any indication of specific carcinogenic factors being involved. It is therefore rational to study this group first. In Table II the figures for the female Group II tumours are given.

TABLE II.—*Group II Tumours in Females.*

| | | Smoking in grams. | | | |
|---|---|---|---|---|---|
| | Non-smokers. | 1–14. | 15–24. | 25 or more. | Total. |
| Lung tumour group | 27 | 9 | 1 | 0 | 37 |
| " Control " (general population) | 28·5 | 7·3 | 1·1 | 0·1 | 37 |

The figures are nearly identical in the two series, as has been the case when other special factors have been examined in the previous studies. The figures clearly show that tobacco smoking, like occupation and area of domicile (urban or rural), is of no importance as an etiological factor in the development of Group II tumours in females.

TABLE III.—*Group II Tumours in Males.*

| | | Smoking in grams. | | | |
|---|---|---|---|---|---|
| | Non-smokers. | 1–14. | 15–24. | 25 or more. | Total. |
| Lung tumour group | 3 | 31 | 6 | 5 | 45 |
| " Control " (general population) | 6·7 | 26·1 | 9·4 | 2·8 | 45 |

Table III shows that also for males there is a very similar situation as regards the smoking level of the Group II lung tumour patients and that of the males of the general population. These figures indicate that in males also tobacco smoking is of no accountable importance for the development of Group II tumours. The similarity in the occurrence of the Group II tumours in the two sexes further substantiates this conclusion.

In Table IV a similar comparison is made between the smoking levels of the males of the general population and that of the male Group I tumour patients.

TABLE IV.—*Group I Tumours in Males.*

| | | Smoking in grams. | | | |
|---|---|---|---|---|---|
| | Non-smokers. | 1.14. | 15–24. | 25 or more. | Total. |
| Lung tumour group | 3 | 123 | 49 | 38 | 213 |
| " Control " (general population) | 28·2 | 128·5 | 41·8 | 14·5 | 213 |

Here a definitely different pattern can be observed, namely, a significantly lower number of non-smokers, and higher number of heavy smokers in the lung tumour group. Even for the other smoking levels the tendency can be observed, and with a systematic deviation towards the extremes.

The figures closely follow the pattern observed by a great number of other students, and the findings definitely point to a close relationship between tobacco smoking and the development of Group I tumours (often designated " epidermoid " tumours) in males.

An extensive review of the main literature in this field in English is given by Doll (1955), and a review of the German literature is given by Randig (1954).

The figures for females are very small, but are nevertheless tabulated, with no comments.

TABLE V.—*Group I Tumours in Females.*

| | | Smoking in grams. | | | |
|---|---|---|---|---|---|
| | Non-smokers. | 1–14. | 15–24. | 25 or more. | Total |
| Lung tumour group | 3 | 1 | 1 | 0 | 5 |
| " Control " (general population) | 4·2 | 0·7 | 0·1 | 0 | 5 |

As the Group I and Group II tumours seem so different in their relation to tobacco smoking, it may be of some interest to compare the two groups, *inter se*, in some more detail. The pertinent figures are given in Table VI.

Our present material has been collected mainly in the period 1951/II to 1955/I, and great efforts have been made to obtain as many cases as possible. The differentiation between Group I and Group II tumours is a comparatively late step in the analysis of each patient. From our previous papers it has been concluded that the two groups most probably are representative for the real occurrence of the histological types for the whole country during the period of this study. We may, therefore, with good reason assume that the relationship between the two groups is also representative.

The non-smoking part of the male population is represented in Table VI by three cases from each group, that is the same number.

TABLE VI.—*Smoking Levels of Group I and Group II Tumour Patients.*

*Males.*

| | Amount smoked in grams. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0. | 1–4. | 5–9. | 10–14. | 15–19. | 20–24. | 25–29. | 30–49. | 50+. | Total. |
| Group I tumours | 3 | 4 | 41 | 78 | 29 | 20 | 15 | 18 | 5 | 213 |
| Group II tumours | 3 | 5 | 14 | 12 | 5 | 1 | 4 | 0 | 1 | 45 |
| Group I tumours associated with tobacco | — | — | 27 | 66 | 24 | 19 | 11 | 18 | 4 | 169 |

*Females.*

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Group I tumours | 3 | 1 | — | — | — | 1 | — | — | — | 5 |
| Group II tumours | 27 | 3 | 3 | 3 | — | 1 | — | — | — | 37 |

If the Group II tumour bearers are representative of the total male population as regards tobacco smoking, and if these tumours have no relation to tobacco smoking, it may be assumed that in the absence of any association between

smoking and lung cancer the remaining smoking part of the male population, like the non-smokers, would produce Group I tumours in a number corresponding to the Group II tumours, at each smoking level.

If these assumptions are accepted, a simple substraction of the Group II tumour cases from the Group I tumour cases, smoking level for smoking level, would reveal the number of Group I tumours associated with tobacco smoking and the number of those not. These figures are shown in Table VI. It seems that in Norway, to-day, approximately one out of five cases of Group I lung tumours in males is not associated with tobacco smoking. This proportion is actually considerably higher than that estimated for England and Wales, since the figure of 17 per cent calculated by Doll (1953) for the age-group 25 to 74 years included the Group II cases and all the female cases. The smallness of the absolute non-smoker figures introduces, however, a certain inexactness in the present study.

TABLE VII.—*Ratio Group I Tumours : Group II Tumours at Different Smoking Levels (Males).*

| | | |
|---|---|---|
| Non-smokers . . . . | 3 : 3 | 1 : 1 |
| Smoking 1– 4 g. . . . | 4 : 5 | 0·8 : 1 |
| „ 5– 9 g. . . . | 41 : 14 | 2·9 : 1 |
| „ 10–19 g. . . . | 107 : 17 | 6·3 : 1 |
| „ 20+ g. . . . | 58 : 6 | 9·7 : 1 |
| „ 30+ g. . . . | 23 : 1 | 23·0 : 1 |

The figures for 20+ and 30+ grams are given alternatively.

If the Group I tumours are compared to the Group II tumours (Table VII), it will be seen that males smoking up to 4 grams of tobacco a day—equivalent to 4 cigarettes—show an occurrence very like that of the non-smokers, that means that they do not develop more Group I tumours than Group II tumours. No relationship to smoking has been registered.

As soon, however, as the amount of smoking is increased above 5 grams a day it will be seen that the ratio Group I : Group II tumours increases steadily as indicated in Table VII. The ratio reaches very impressive figures when the limit of 30 grams a day has been passed.

The figures not only strongly indicate a relationship between Group I lung carcinomas and smoking, but they also give an indication of the relative risks associated with the different smoking levels. The figures are of the same order of magnitude as those reported by Doll and Hill (1952), Levin (1954), Randig (1954) and Hammond and Horn (1955).

Whereas previous students of this problem have for the most part based their conclusions upon the occurrence of all types of lung cancer and made their calculations on the basis of the population in general, the present study has utilized a totally different material for comparison. Here the "control" material also consists of lung tumours, originally from the same sources as the material proper and only separated from this through a histological analysis. This "control" material comes closer to the ideal material than any other, as defined by Kreyberg (1954). The present findings, therefore, strengthen considerably the conclusions of previous researchers. It would be strange if another underlying primary factor should be the real cause, and that the use of tobacco and the development of lung cancer were two quantitatively corresponding manifestations of such an unknown factor. At least, no such factor has been found, or even reasonably suggested.

The objection that the difference in age representation in the two tumour groups may explain the different smoking figures is probably not valid. In the Norwegian population smoking is more prevalent among the younger people than among the older. If, therefore, our Group II had comprised adenocarcinoma patients only, a greater number of non-smokers and light smokers might have been expected in that group, because adenocarcinomas increase markedly with advancing age. But the adenomas and the salivary gland tumours bring into the Group II a considerable number of younger people. If anything, the bias would rather go in the direction of more smoking in Group II than in Group I. To test the validity of this standpoint a survey has been made of the male Group I and Group II tumour patients, including the ages 45 to 64 years only. In the Appendix, Table II, the pertinent figures are given, in relation to our original " control " material, with due corrections for the age representation, as used in Tables III and IV. Essentially the same picture is maintained.

The importance of the close association with four-fifths of the Group I tumours in males to tobacco smoking should, however, not lead to a neglect of the other possible factors in the carcinogenic situation.

In a previous paper (Kreyberg, 1954*c*) a general air pollution was not found acceptable as a primary, or a secondary, factor in the recent increase in lung cancer, because the female part of the population, breathing the same air, seems unaffected.

In another paper (Kreyberg, 1954*d*) a greater tendency to Group I lung cancer in males was observed in individuals with " dusty " occupations and trades, as well as in " clerical " and " professional " workers, when compared to individuals with " open air " and " house " work. In that paper it was underlined that the dust, *per se*, might be of importance, but it was also mentioned that other factors, in the line of life habits, more or less closely associated with different occupations, might be involved. Among the life habits tobacco smoking holds a prominent place.

In Table VIII a survey is given, in grams, of the smoking levels in the different occupations in the Group I tumours in males, arranged also according to domicile. The material is, admittedly, small and difficult to analyse. It seems, however, that at least there is no indication that the comparatively higher occurrence of Group I tumours in males in " dusty " trades and occupations is associated with an especially high smoking level. The figures may rather be interpreted as showing that " dusty " occupations may add another carcinogenic insult to the effect of the smoking. It cannot therefore theoretically be denied that besides the well known lung carcinogenic agents, such as nickel, asbestos, coal tar and uranium ore, some other " dusts " may be active and of importance in the development of some cases of lung cancer, even if the agent is so weak as not to be easily discovered. Exposure to metal dusts may especially be noted. It is worth noting, however, that among the 92 cases in " dusty " occupations there is not a single non-smoker.

" Clerical " and " professional " workers in the male Group I tumour material seem to be rather heavy smokers. Not one smoked less that 5 grams, and only 8 out of 53 patients smoked less than 10 grams a day. Non-smokers were not found.

The " open air " and " house " workers represent the largest occupational category, but they show the lowest number of Group I tumour cases—44 cases in all. It seems that also the tobacco smoking is on a lower general level than in the other occupational categories. This holds good not only for the relative number of smokers, but also for the amount smoked. Furthermore, it is interesting to note that

TABLE VIII.—*Smoking Levels in the Different Occupational Categories and in the Different Types of Domicile in Group I Tumour Patients. Males.*

| | Amount tobacco smoked in grams. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0. | 1–4. | 5–9. | 10–14. | 15–19. | 20–24. | 25–29. | 30–49 | 50 +. | Total. |
| *Countryside* | | | | | | | | | | |
| "Open air" activities and "house" work | 3 | — | 3 | 3 | 1 | 1 | 2 | — | — | 13 (10) |
| "Clerical" and "professional" work | — | — | 1 | 3 | 2 | 1 | — | — | — | 7 |
| "Dusty" occupations | — | — | 3 | 4 | 1 | 2 | 1 | — | — | 11 |
| | 3 | — | 7 | 10 | 4 | 4 | 3 | — | — | |
| *Non-industrial towns* | | | | | | | | | | |
| "Open air" activities and "house" work | — | — | 2 | 7 | — | 1 | 1 | 1 | 1 | 13 |
| "Clerical" and "professional" work | — | — | 1 | 2 | 1 | 1 | 1 | 4 | — | 10 |
| "Dusty" occupations | — | — | 7 | 7 | 2 | 1 | 2 | 3 | — | 22 |
| | — | — | 10 | 16 | 3 | 3 | 4 | 8 | 1 | |
| *Industrial towns and centres* | | | | | | | | | | |
| "Open air" activities and "house" work | — | 1 | — | 3 | — | — | — | — | — | 4 |
| "Clerical" and "professional" work | — | — | — | 1 | 3 | 1 | 1 | 1 | — | 7 |
| "Dusty" occupations | — | 1 | 3 | 7 | 2 | 1 | — | 1 | — | 15 |
| | — | 2 | 3 | 11 | 5 | 2 | 1 | 2 | — | |
| *Larger towns (Oslo, Bergen, Trondheim)* | | | | | | | | | | |
| "Open air" activities and "house" work | — | 1 | 3 | 5 | 3 | 1 | 1 | — | — | 14 |
| "Clerical" and "professional" work | — | — | 6 | 4 | 9 | 2 | 2 | 4 | 2 | 29 |
| "Dusty" occupations | — | 1 | 7 | 23 | 3 | 5 | 2 | 2 | 1 | 44 |
| | — | 2 | 16 | 32 | 15 | 8 | 5 | 6 | 3 | |

all the 3 non-smokers in the total material of male Group I tumour patients belong to this category. The lung history of these 3 cases is as follows;

(1) Farmer, 75 years old (Hordaland Fylke). No serious lung symptoms until two years before his death from a squamous cell carcinoma of the lung.

(2) Farmer, 67 years old (Hedmark Fylke). Since 1914 suffering from asthma with constant use of adrenaline. In 1951 a short story of increased lung symptoms, with the subsequent diagnosis of a squamous cell carcinoma.

(3) Fisherman, 67 years old (Akershus Fylke). During the winter of 1953 some dry coughing, in March pains and haemorrhage, in May diagnosis of a squamous cell carcinoma.

It may also be worth noting that all these patients belonged to age-groups above that with the maximum occurrence of Group I tumour cases.

Sailors and patients with unknown domicile have not been included in this part of the analysis.

In this connection it may be of interest to recall that in the study of the geographical distribution of the Norwegian lung cancer material, it was observed that Group I tumours were more heavily represented in the "smaller non-industrial

towns " with " fresh air ", than in the " smaller industrial towns and centres " with more or less polluted air. This finding was initially unsuspected by the writer, on the background of the predominant assumption that general air pollution is of some importance for the development of lung cancer. The original paper comprised then 235 cases of lung cancer. The figures of the present material, augmented to 300 cases, confirm the previous observation.

(10) The original " control " material did not give any information as to smoking habits in different types of domicile, although the finding of more moderate smoking in rural districts was expected. After the publication of the paper on geographical distribution, some other material with a bearing on the Norwegian smoking habits was placed at my disposal through the kindness and the generosity of " Fakta ", Oslo, an institute for marketing research. This institute had just completed a survey for one of our tobacco factories, and in the Appendix, Table III, some data are recorded, and in the text Fig. 1 presents the picture of the total smoking as well as the different types of smoking in areas corresponding to our " Rural districts ", "Smaller non-industrial towns ", " Smaller industrial towns and centres " and " Larger towns ". The figures presented are not comparable to the figures of our original " control " material, because of different criteria used. In our original " control " material, as well as in our lung cancer material, a smoker was defined as " a person who has smoked as much as 1 g. of tobacco (in any form) daily for at least one year ", whereas in the " Fakta " material, only present smokers are regarded as smokers. This will explain the very important differences as regards the percentages of smokers and non-smokers in the two series. The smoking types in the different geographical areas of the " Fakta " material are, however, comparable *inter se*, and Fig. 1 shows that the smoking habits in the smaller towns differ markedly. The percentage of smokers is practically the same, but the " Smaller non-industrial towns ", which mainly represent commercial and administrative centres, show a considerably higher number of pure and mixed cigarette smokers than the " Smaller industrial towns and centres ", even if the average number of grams smoked is the same (Appendix, Table III). The male Group I tumour patients show a heavier type of smoking in the former, as compared to the latter, as will be seen from Table VIII.

In Table IX the types of smoking are given for the male Group I and Group II tumour patients. There is a heavier representation of pure and mixed cigarette

TABLE IX.—*Different Smoking Types of Group I and Group II Tumour Patients (Males).*

| Type of smoking. | Amount smoked in grams. 0. | 1–4. | 5–9. | 10–14. | 15–19. | 20–24. | 25–29. | 30–49. | 50 +. | Total. |
|---|---|---|---|---|---|---|---|---|---|---|
| **Group I.** | | | | | | | | | | |
| Non-smokers | 3 | — | — | — | — | — | — | — | — | 3 |
| Pure pipe smokers | — | 3 | 11 | 19 | 1 | 2 | — | — | — | 36 |
| Pipe and cigarettes | — | — | 12 | 18 | 11 | 4 | 5 | 3 | 4 | 57 |
| Pure cigarettes | — | 1 | 18 | 41 | 17 | 14 | 10 | 15 | 1 | 117 |
| **Group II.** | | | | | | | | | | |
| Non-smokers | 3 | — | — | — | — | — | — | — | — | 3 |
| Pure pipe smokers | — | — | 6 | 4 | 1 | 1 | 1 | — | — | 13 |
| Pipe and cigarettes | — | — | 3 | 2 | 1 | — | 1 | — | — | 7 |
| Pure cigarettes | — | 5 | 5 | 6 | 3 | — | 2 | — | 1 | 22 |

smokers in the Group I series, and more pipe smokers in the Group II. The Group I series, as regards cigarette smoking, shows considerably higher figures than the Norwegian " control " material presented by Kreyberg (1954). A detailed comparison with the " Fakta " material is useless because of the different criteria used for

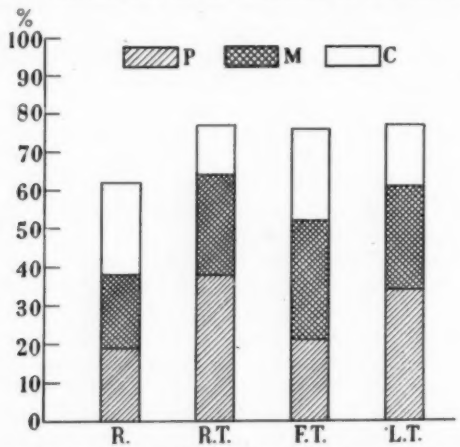


FIG. 1.—The smoking habits of males of different types of domicile, " Fakta " material. The columns show percentage smokers in total, as well as separately for pure pipe smokers (P), mixed pipe-cigarette smokers (M) and pure cigarette smokers (C), in rural districts (R), smaller non-industrial towns (RT), smaller industrial towns and centres (F.T) and larger towns (L.T). P ; M ; C .

non-smokers. If one examines the ratio Group I : Group II tumours in pure pipe smokers, pipe and cigarette smokers and pure cigarette smokers, one finds figures as shown in Table X.

TABLE X.—*Ratio Group I : Group II Tumours Males. Different Types of Smoking.*

| Amount smoked. | None. | Pipe only. | Pipe and cigarettes. | Cigarettes only. |
|---|---|---|---|---|
| Under 10 g. | — | 2·3 : 1 | 4·0 : 1 | 1·9 : 1 |
| 10–19 „ | — | 4·0 : 1 | 9·7 : 1 | 6·4 : 1 |
| 20 + „ | — | 1·0 : 1 | 16·0 : 1 | 13·3 : 1 |
| Total | 1 : 1 | 2·8 : 1 | 6·0 : 1 | |

The absolute figures are small and the picture is not completely clear, therefore no precise conclusions can be drawn, but it may be worth calling attention to the seemingly lacking increase in the ratio of the pure pipe smokers to the increasing amount of tobacco smoked, in contrast to the definite and systematic increase in the ratio when cigarette smoking is involved. This may point in the direction that pure pipe smoking actually is connected with a more moderate risk, as regards lung cancer development, than is cigarette smoking. Furthermore, on the average the pure pipe smokers do not seem to use as great amounts of tobacco as do the cigarette smokers, and a quantitative factor may possibly also be of importance in this respect.

One feature from the tables reproduced is very striking, namely, the different occurrence of some types of lung cancer in the two sexes, even among the non-smokers. As there are many more non-smokers among the females, and as many female smokers are very moderate smokers, the absolute figures cannot be compared. But, if the ratio Group I tumours to Group II tumours is again used as an analytical method on the male and female non-smokers, one finds the figures 1 : 1 in males (3 : 3 cases), and 1 : 9 in females (3 : 27 cases) (Table XI).

If the figures for smokers at the level of less than 5 grams, where no carcinogenic association has been observed in our material, are also included, essentially the same picture is found ; a ratio for males 1 : 1·1 (7 : 8 cases), and for females 1 : 7·5 (4 : 30 cases). The basic figures are found in Table VI.

TABLE XI.—*Sex Differences. Ratio Group I : Group II Tumours.*

| | | |
|---|---|---|
| Male non-smokers . . . . | 1 : 1 | (3 : 3) |
| „ smokers . . . . | 5 : 1 | (210 : 42) |
| Female non-smokers . . . . | 1 : 9 | (3 : 27) |
| „ smokers . . . . | 1 : 5 | (2 : 10) |

With due allowance for the smallness of the numbers, this shows that even in non-smokers the males dominate the Group I tumours markedly. Wynder (1954), in his Table VI presents—from his own material and from the literature—a number of figures showing the occurrence of types of lung cancers (very much like our own grouping) in non-smoking males, and from that material one arrives at a ratio 2·4 : 1, a very low ratio, even if not as low as the Norwegian one quoted above.

The ratio " Epidermoid " carcinomas (including " oat-cell " carcinomas) ; " Adenocarcinomas " (including bronchiolar cell carcinomas) from Wynder's collected material differs from my present material, the explanation being that adenomas and salivary gland tumours are also included in my Group II tumours. If these are excluded, my material shows a ratio 1·8 : 1, very close to Wynder's.

In addition, the ratios are given from four Norwegian post-mortem series, partly from the periods when the new carcinogenic situation was not yet manifested (Table XII). In this table all cases are included, smokers as well as non-smokers. The different figures quoted seem altogether to strengthen the above conclusion that, in Norway at present, the ratio Group I : Group II tumours in male non-smokers is very nearly 1 : 1.

TABLE XII. *Ratio Group I : Group II Tumours in Males in Four Norwegian Post-mortem Series.*

| Material. | Number of cases. | | Ratio. |
|---|---|---|---|
| Christiansen I R. H. (1925–44) . . | 25 | 15 . | 1·7 : 1 |
| Jacobsen Ullevål (1937–46) . . | 46 | 24 . | 1·9 : 1 |
| Christiansen II R. H. (1945–52/1) . | 40 | 14 . | 2·9 : 1 |
| Kreyberg (1948–55) . . . | 213 | 45 . | 4·7 : 1 |

These figures may be supposed to be in contrast to the statement by Doll (1953); " That the incidence of lung cancer in non-smokers may be the same in men and in women and in residents in areas of different density of population," a statement with which Wynder agrees. Our Norwegian figures may initially seem more in

agreement with the standpoint of Fischer (1954/55), who comments upon Doll's conclusion as follows ; " I have the opinion, from the critically digested available information, that such a conclusion cannot be drawn."

Possibly the contrasting statements may be reconciled. It should be emphasised that the very marked sex difference in the Norwegian material just mentioned concerns the Group I tumours only. If the figures from Table XI are used to compare all types of lung cancer, seen as an entity—and that is the background of Doll's analysis—one finds a total of 6 " lung cancers " (3 + 3) in the non-smoking males and 30 cases (3 + 27) in the non-smoking females. As the non-smoking female part of the population however is very much greater than the corresponding male part, a factor of 5 is very likely according to our previously quoted figures, one arrives at an approximately equal sex occurrence of " lung cancer " total among non-smokers. As Group I tumours in the Norwegian material are rather uncommon among females at all, as well as among non-smoking males, the peculiar sex difference in the occurrence in the Group I tumours is easily obscured by the great number of Group II tumours if special attention is not paid to the histological typing, followed by a separate treatment of the two groups. Again, it seems that the value of this analytical procedure, when fully used, has been demonstrated.

The enigma of the preponderance of male victims of Group I tumours in non-smokers is further emphasized in the Norwegian material by the fact that all cases (admittedly only 3) were among the " open air " workers (2 farmers and 1 fisherman), with not a single case of Group I tumours belonging to the categories " dusty ", " clerical " or " professional " workers.

This means that the possibility of a special sex disposition to the development of Group I tumours in males cannot be denied. Whether this disposition is based upon architectural differences in the gross anatomy of the lungs, upon different biological responses of the epithelium, or upon hormonal factors is completely unknown, and the question is at present unassailable by clinical and experimental methods. Gross anatomical studies however, are possible.

Such a sex factor may even be operative in cases where a most probable special causative factor has been found, as seems to be the case regarding tobacco smoking. From the literature it seems that the decided increase in male lung cancers, and that means Group I tumours, started at a time when cigarette smoking was not very prevalent. Considering a latency period, or a period of development, of some 20 to 40 years, the changed carcinogenic situation should, accordingly, have commenced at the end of the last, or at the very beginning of this century for a series of countries. If the female response to tobacco smoking is the same as that of the male it would accordingly be reasonable to-day to expect the finding of a definite increase in female Group I tumours in the countries leading in this respect. A critical study of the literature does not reveal with certainty that such an increase has actually taken place.

## SUMMARY AND CONCLUSIONS

In a series of previous papers it has been shown that an increase in lung cancer in males has taken place in Norway in this century, closely following the pattern of development in a number of other countries, although, it seems, with the delay of a decade or two.

In the beginning of this century a slow and parallel rise in the number of cases of lung cancer registered in males and females was found, but gradually, and from

the middle of the nineteen forties especially, the increase in male cases has greatly exceeded the increase in female cases.

A study of the histological types of lung cancer has revealed that from the period of the more pronounced increase in male cases, the relative frequency of the histological types has also changed, with a steadily increasing number of Group I tumour cases (squamous cell, large cell and small cell carcinomas) in males. No such change has been found in females. It has previously been concluded that nearly the whole increase in female cases, and part of the increase in male cases, which has been registered, is caused by better diagnostic facilities, whereas the specific increase in Group I tumour cases in males is caused by a real increase in the development of lung tumours. This means that a new carcinogenic situation has been established, which in Norway manifested itself definitely in the middle of the nineteen forties. As the Group I tumour cases in males have a period of development ranging between twenty and forty years or more, the new carcinogenic situation should, therefore, have commenced early in this century.

This new carcinogenic situation, up to the present moment, has been manifested through male victims only. From theoretical considerations, as well as from our special studies, it is regarded as unlikely that this new carcinogenic situation is based upon a general air pollution, even if its effects are mainly observed in urban areas. The main active principle(s) should be sought in the closer environments of the males, most probably in connection with the male's working conditions and/or his life habits.

Whereas it is well known that certain occupations, connected with radio-active material, nickel, asbestos and coal tar, are associated with an increased lung cancer development, the Norwegian material does not show any special trade or occupation with such a marked risk that the increased occurrence of Group I tumour cases in males can be explained solely by occupational hazards.

Admittedly males in " dusty " occupations generally have an increased lung cancer frequency as compared to " open air " workers. But nearly as great a risk is also noted among " clerical " and " professional " workers.

A study of the tobacco smoking habits of the lung cancer cases has now been conducted in such a manner that the habits of the general population, the habits of the Group I tumour cases, as well as those of the Group II tumour cases (adenocarcinomas, bronchiolar cell carcinomas, adenomas benign and malignant, and salivary gland tumours of the lungs), have been analysed separately for each group and for each sex, and the figures compared.

The material has consisted of 213 Group I and 45 Group II tumour cases in males, and 5 Group I and 37 Group II tumour cases in females. The main conclusions of this study are as follows ;

(1) As no differences have been found, either in males or in females, between the smoking habits of the Group II tumour patients and the smoking habits of the population in general, it is concluded that tobacco smoking has no relationship to the occurrence of Group II lung tumours. Such tumours represent, in Norway to-day, nearly 90 per cent of all female lung tumour cases, but less than 20 per cent of all male cases.

(2) As a considerably lower number of non-smokers are found among the male Group I lung tumour patients than among the male Group II tumour patients, as well as among the corresponding male " control " material, and as a consistently and steadily increasing ratio of Group I : Group II cases is observed with the

increasing amounts of tobacco smoked, it is concluded that tobacco smoking is closely related to the development of a considerable proportion of the Group I tumour cases in males. The very limited female material does not present any contradictory facts invalidating this conclusion.

(3) As a certain number of Group I tumour cases occur in male and in female non-smokers, it is concluded that not all Group I tumour cases are caused by, or are influenced in, their development by tobacco smoking.

(4) From an analysis of the ratio Group I : Group II tumours in males, it has been calculated that, in Norway at present, four out of five cases of Group I lung tumours in males are related to tobacco smoking, and that one out of five cases arises from causes unrelated to tobacco smoking.

(5) As males in " dusty " work show the relatively greatest number of Group I lung tumours in spite of a more moderate tobacco consumption than shown by the Group I lung tumour patients among " clerical " and "professional " workers, it may tentatively be suggested that industrial dusts and fumes add an aggravating factor to the injury caused by tobacco smoking, as regards the development of lung cancer.

(6) A previously recorded finding of relatively more Group I tumour cases in males in smaller non-industrial towns (administrative and commercial centres) with " fresh air ", than in smaller industrial towns and centres, with more, or less, polluted air, may be explained by the finding of more pure and mixed cigarette smokers in the former as compared to the latter type of community.

(7) The relationship between pipe smoking and lung cancer development is much less marked than the relationship between cigarette smoking and lung cancer.

(8) The ratio Group I : Group II tumours in non-smokers is nine times greater in the males than in the females. This very great difference, *per se*, as well as the fact that the only three male Group I tumour non-smokers, registered in the present material, occurred among the " open air " workers, makes it difficult to ascribe the whole sex difference in the occurrence of Group I tumours to external factors alone. A biological sex difference, architectural and/or biochemical, influencing the response of the lungs to some, or all, of the principles causing lung cancer, cannot be excluded.

After this discussion, and after the conclusions presented, it may be useful to underline the following points.

Firstly, the conclusions are based upon a special histological subdivision of the " lung cancer " material, according to certain definite criteria. If this grouping and these criteria used are not followed the same results cannot be obtained. An off-hand denial of the possibility of making a sufficiently precise histological grouping is dismissed by the results. Not every case can be grouped with certainty, but most cases can.

Secondly, the results of the present study are so systematic and so clear that it is unreasonable not to consider the findings as expressions of true conditions. An artefact with such a degree of consistency is most improbable.

Thirdly, the figures quoted represents trends and relationships only. They do not express fixed mathematical correlations generally applicable. The figures are approximate and applicable to the conditions in Norway during the period of investigation, and cannot be applied to other countries and to other conditions without further qualifications. The trends of the general conclusions I do, however, regard as generally valid.

The present investigation has been aided by a generous grant from " Tobaksfabrikermes Landsforening av 1901 "

My sincere thanks are also due to the " Fakta " Marketing Research Institute, Oslo, for valuable statistical material, as regards smoking habits in Norway to-day.

I am likewise greatly indebted to Dr. R. Doll, London, and Dr. R. Korteweg, Amsterdam, for valuable discussions during the preparation of the manuscript.

## REFERENCES

BRESLOW, L., HOAGLIN, L., RASMUSSEN, G. AND ABRAMS, H.—(1954) *Amer. J. Publ. Hlth.*, **44**, 171.

DOLL, R.—(1953) *Brit. J. Cancer*, **8**, 303.—(1955) ' Advances in Cancer Research.' New York (Academic Press Inc.).

*Idem* AND HILL, A. B.—(1952) *Brit. med. J.*, ii, 1271.

FISCHER, W.—(1954/55) *Wiss. Z. Fried. Schiller Universitaet Jena*, **4**, 21.

HAMMOND, E. C. AND HORN, D.—(1955) Stenciled report from the Annual Meeting of the American Medical Association in Atlantic City, June 6.

KREYBERG, H. J. A.—(1954) *Brit. J. Cancer*, **8**, 13.

KREYBERG, L.—(1952) *Ibid.*, **6**, 112.—(1954*a*) *Ibid.*, **8**, 199.—(1954*b*) *Ibid.*, **8**, 209.—(1954*c*) *Ibid.*, **8**, 599.—(1954*d*) *Ibid.*, **8**, 605.

LEVIN, M. L.—(1954) *N.Y. St. J. Med.*, **54**, 769.

LICKINT, F.—(1953) ' Ätiologie und Prophylaxe des Lungenkrebses.' Dresden und Leipzig (Steinkopff).

RANDIG, K.—(1954) *Öff. GesundhDienst.*, **16**, 305.

WYNDER, E. L.—(1954) *Penn. med. J.*, **57**, 1073.

*Idem* AND GRAHAM, E. A.—(1950) *J. Amer. med. Ass.*, **143**, 329.

APPENDIX TABLE I.—*Smoking Habits of the " General Population ", Based upon an Aggregation of Groups* 1, 2, 4, 5, 6 (*M*) *and Groups* 1, 2, 3, 4, (*F*), *Excluding Persons* 85 *Years and Older, from Kreyberg* (1954).

*Males.*

| | Non-smokers. | | Smoking in grams. 1–14. | | 15–24. | | 25 or more. | | Total. | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age. | No. | Per cent. | No. | Per cent. | No. | Per cent. | No. | Per cent. | No. | Per cent. |
| 35 | 268 | 20·92 | 716 | 55·89 | 240 | 18·74 | 57 | 4·45 | 1281 | 100·00 |
| 35–44 | 176 | 14·03 | 680 | 54·23 | 322 | 25·68 | 76 | 6·06 | 1254 | 100·00 |
| 45–54 | 102 | 10·66 | 520 | 54·34 | 263 | 27·48 | 72 | 7·52 | 957 | 100·00 |
| 55–64 | 58 | 13·33 | 272 | 62·53 | 77 | 17·70 | 28 | 6·44 | 435 | 100·00 |
| 65–84 | 40 | 17·32 | 153 | 66·23 | 23 | 9·96 | 15 | 6·49 | 231 | 100·00 |
| Total | 644 | | 2341 | | 925 | | 248 | | 4158 | |

*Females.*

| | Non-smokers. | | Smoking in grams. 1–14. | | 15–24. | | 25 or more. | | Total. | |
|---|---|---|---|---|---|---|---|---|---|---|
| Age. | No. | Per cent. | No. | Per cent. | No. | Per cent. | No. | Per cent. | No. | Per cent. |
| 35 | 237 | 58·52 | 151 | 37·28 | 17 | 4·20 | 0 | 0·00 | 405 | 100·00 |
| 35–44 | 124 | 60·79 | 66 | 32·35 | 13 | 6·37 | 1 | 0·49 | 204 | 100·00 |
| 45–54 | 131 | 69·31 | 50 | 26·46 | 6 | 3·17 | 2 | 1·06 | 189 | 100·00 |
| 55–64 | 111 | 87·40 | 13 | 10·24 | 3 | 2·36 | 0 | 0·00 | 127 | 100·00 |
| 65–84 | 54 | 90·00 | 6 | 10·00 | 0 | 0·00 | 0 | 0·00 | 60 | 100·00 |
| Total | 657 | | 286 | | 39 | | 3 | | 985 | |

APPENDIX TABLE II.—*Smoking Levels of Male Group I and Group II Tumour Patients (Age-Group 45–54 and 55–64 only) and "Controls" Matched to Age-Groups.*

| | Non-smokers. | Smoking in grams. 1–14. | 15–24. | 25 +. | Total cases. |
|---|---|---|---|---|---|
| Group I | 0 | 91 | 40 | 30 | 161 |
| "Control" (general population) | 19·5 | 94·9 | 35·4 | 11·2 | 161 |
| Group II | 1 | 15 | 2 | 4 | 22 |
| "Control" (general population) | 2·6 | 12·9 | 4·9 | 1·6 | 22 |

APPENDIX TABLE III.—*Norwegian Smoking Habits.*

(From a market analysis by "Fakta")

| | Rural districts. | Smaller non-industrial towns. | Smaller industrial towns and centres. | Larger towns. |
|---|---|---|---|---|
| *Males.* | | | | |
| Number examined | 643 | 264 | 279 | 783 |
| Per cent smokers (total) | 62 | 77 | 76 | 77 |
| „ pipe smokers | 24 | 13 | 24 | 16 |
| „ mixed cigarette smokers | 19 | 26 | 31 | 27 |
| „ pure cigarette smokers | 19 | 38 | 21 | 34 |
| Average number grams per day per smoker | 8·6 | 10·7 | 10·6 | 10·3 |
| Per cent smokers each age group: | | | | |
| 30 years and younger | 74 | 91 | 88 | 79 |
| 30–44 years | 72 | 90 | 79 | 81 |
| 45–59 years | 51 | 62 | 69 | 80 |
| 60 years and more | 49 | 70 | 65 | 63 |
| *Females.* | | | | |
| Number examined | 723 | 262 | 278 | 833 |
| Per cent smokers (all cigarette) | 10 | 31 | 25 | 35 |
| Per cent smokers each age group: | | | | |
| 30 years and younger | 28 | 45 | 26 | 35 |
| 30–44 years | 13 | 37 | 39 | 45 |
| 45–59 years | 2 | 24 | 14 | 29 |
| 60 years and more | 2 | 7 | — | 12 |

# BRONCHIAL CARCINOMA: A STATISTICAL STUDY OF 741 NECROPSIES WITH SPECIAL REFERENCE TO THE DISTRIBUTION OF BLOOD-BORNE METASTASES.

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ADVANCES in treatment have made possible the local eradication of many primary malignant tumours; in contrast, little progress has been made with the much more difficult problems of influencing blood stream spread or of dealing effectively with established multiple metastases. Except for the temporary control by alteration in hormone balance of a few special types of tumour little of value to the patient has yet come from much detailed fundamental research work on the chemical control of cancer. It would seem, therefore, that one of the most important investigations in clinical cancer research at the present time concerns those factors which determine the production and localisation of metastases.

The reasons for the observed distributions of blood-borne metastases have been a matter of controversy for many years. At one extreme we have the view that cells from certain invasive tumours are commonly released into the blood stream at an early stage and that whether metastases then develop and what their number and distribution may be, depends on the nature of the tumour cells and the suitability of the tissues in which they lodge as an environment for this growth. At the other extreme it is held that the entry of tumour cells into the blood stream depends largely on differences in tissue tensions and that if they do get into the circulation, the distribution of the resulting metastases will depend on the size of the cell clumps, the relative sizes of the organs and the state of their capillaries. The first may be called the "seed and soil" theory and the second the "mechanical" theory. It is almost certain that the truth is a variable and complicated association of the two.

When animals have tumour suspensions injected into their arterial circulations, each tumour tends to produce its own metastatic pattern, though metastases are frequently found in some organs whatever tumour suspension is used (Sugarbaker, 1952). If care is taken to inject tumour suspensions simultaneously into the blood supply of the liver and the lung so that metastases become established at the same time in both, the liver tumours tend to grow faster than the lung tumours. This suggests that for these two organs and for some tumour types at least, tissue environment is an important factor (Lucke, Breedis, Woo, Berwick and Nowell, 1952).

It is only too obvious that some types of tumour metastasise more readily than others, but it is not so clear whether this property depends upon their inherent invasiveness, alterations in neighbouring tissue restraints, the tissue pressures exerted (Young and Griffith, 1950), or the elaboration by the tumour cells of enzymes which cause connective tissue destruction. It seems certain that there is much more tumour cell dissemination than metastasis and that many

cells are destroyed in the blood stream. Tumour cells can be seen in lung capillaries (Willis, 1952) and in sternal bone marrow (Kreyberg and Poppe, 1940); they can pass immediately through the pulmonary circulation in rabbits and rats at least (Zeidman and Buss, 1952) and may be shunted from the systemic to the vertebral venous system (Batson, 1940, 1942; Anderson, 1951). It is quite possible that they may also travel by other by-passing mechanisms such as those which operate in shock (Trueta *et al.*, 1947). There seems to be evidence that any part of the body may be presented with tumour cells from any primary site.

If we accept the hypothesis of general blood stream dissemination, the mechanical factors determining the distribution of metastases may be: the size of the tumour fragments (Zeidman, McCutcheon and Coman, 1950), whether the tumour cells occur in clumps or singly (Watanabe, 1954), their number, the state of the capillaries through which they pass, and the rate of blood flow within them. There is in fact no close correlation between the number of tumour cells reaching an organ and the number of metastases, but there is an excellent correspondence between the number lodged in capillaries and the number of secondary deposits (Coman, de Long and McCutcheon, 1951). The various organs of the body in rats seem to offer little resistance to the growth of tumour implants (Baxter, Leupold-Loewenthal and Burton, personal communication). Some tumours grow readily even in spleen and muscle (de Long and Coman, 1950), a fact which supports the contention that much depends on the number of cells which become arrested in an organ, and on the ease with which they can escape from the blood vessels.

Primary tumours of the lung would seem to form a good group for this type of clinical study, since for each tumour the chance of even dissemination throughout the blood stream is likely to be greater than for most other sites. We have therefore analysed the distribution of metastases found at necropsy in eight London hospitals; four teaching hospitals and four Regional Board hospitals, to see if we can by this means present any useful facts and draw any interesting conclusions which might help towards the elucidation of this most important aspect of the cancer problem.

*Source of the material*

Details of 741 patients, who died with carcinoma of the bronchus in the years 1948–52, have been collected from the records of eight London hospitals. All these patients were subjected to careful clinical study during the course of their disease and a complete necropsy was performed in all but 94 cases where no intracranial examination was carried out.

During the same period the total number of necropsies performed at the eight hospitals was 10,430. Table I shows how this number was made up and what proportion of all necropsies related to patients who had died from carcinoma of the bronchus. In the case of the Royal Marsden Hospital this proportion is rather high (12·9 per cent) no doubt due to the special nature of its work and to its close association with the Brompton Hospital for Diseases of the Chest. If we exclude the Royal Marsden Hospital, 6·8 per cent of all necropsies related to patients who had died from carcinoma of the bronchus.

This percentage agrees with the figure of 6·9 per cent found in the British Empire Cancer Campaign's (1950) survey of the records of several London hospitals for for the years 1945–49. It is, however, much higher than either the 2·3 per cent

TABLE I.—*Sources of Material and Proportions of All Necropsies Performed on Patients who Died from Bronchial Carcinoma.*

| Hospital. | All necropsies 1948–52. | Number referring to carcinoma bronchus. | Per cent carcinoma bronchus. | Number with full necropsy including intracranial examination. |
|---|---|---|---|---|
| Hammersmith Hospital | 1975 | 156 | 7·9 | 122 |
| St. Mary Abbots Hospital | 2016 | 113 | 5·6 | 90 |
| Middlesex Hospital | 1466 | 87 | 5·9 | 58 |
| St. George's Hospital | 1365 | 87 | 6·4 | 84 |
| Archway Hospital | 1034 | 83 | 8·0 | 82 |
| St. Mary's Hospital, Islington | 1424 | 99 | 7·0 | 99 |
| Highgate Hospital | 652 | 52 | 8·0 | 51 |
| All hospitals except Royal Marsden Hospital | 9932 | 677 | 6·8 | 586 |
| Royal Marsden Hospital | 498 | 64 | 12·9 | 61 |
| All hospitals including Royal Marsden Hospital | 10,430 | 741 | 7·1 | 647 |
| Four Teaching Hospitals | 5304 | 394 | 7·4 | 325 |
| Four Regional Board Hospitals | 5126 | 347 | 6·8 | 322 |

found by Heady and Kennaway (1949) or a similar figure found by Steiner, Butt and Edmondson (1950).

The patients in this series died with the disease in varying stages of development. Many of the patients (64 per cent*) had received no specific treatment and the remainder had been treated by a variety of methods. While therapeutic intervention may have influenced the spread of malignant emboli, many of the comparatively small number of treated patients had obvious metastases before treatment started.

The analysis of data obtained in this way, by the retrospective examination of records from a variety of sources, is fraught with difficulties. It cannot be expected to replace or approach in accuracy a properly planned laboratory experiment or a well designed clinical investigation. Certain suggestions or hypotheses may, however, emerge which might later be tested by more direct and controlled methods.

*Features of the material*

Before considering that part of the data which relates particularly to metastases it is important to examine in more detail the constitution of the series as a whole with special reference to factors such as sex, age and histology. By attending especially to differences between hospitals we may by this means learn something of selection processes and individual pathological interpretations which, in turn, may be useful when we come to study the data relating to metastases.

(i) *Sex.*—Table II shows the material analysed according to sex and age for each hospital. The proportions of females range from 14 per cent at St. Mary

* 313 out of 487 patients in respect of whom we have a definite record. This does not include the treatment records of the three wings of the Whittington Hospital, namely, Archway, St. Mary's Islington, and Highgate.

TABLE II.—*Sex and Age Distribution of Material by Hospitals.*

| Hospital. | Sex. | Age (years). 20– | 30 | 40– | 50– | 60– | 70– | 80– | Not stated. | Total. | Per cent females. | Mean age (years). | S.E. of mean (years). |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (*a*) Hammersmith Hospital | M | — | 5 | 21 | 40 | 52 | 9 | 1 | — | 128 | 18 | 58·5 | 0·82 |
| | F | — | 3 | 3 | 10 | 5 | 5 | 2 | — | 28 | | | |
| (*b*) St. Mary Abbots Hospital | M | — | 3 | 10 | 25 | 34 | 22 | 3 | — | 97 | 14 | 63·0 | 0·83 |
| | F | — | 1 | — | 3 | 6 | 3 | 3 | — | 16 | | | |
| (*c*) Middlesex Hospital | M | — | 2 | 6 | 32 | 24 | 3 | — | 1 | 68 | 22 | 56·6 | 0·97 |
| | F | — | 4 | 4 | 4 | 6 | 1 | — | — | 19 | | | |
| (*d*) St. George's Hospital | M | — | 1 | 18 | 25 | 25 | 4 | — | — | 73 | 16 | 56·8 | 1·03 |
| | F | — | 2 | 2 | 4 | 4 | 1 | 1 | — | 14 | | | |
| (*e*) Royal Marsden Hospital | M | 1 | 4 | 8 | 16 | 22 | 1 | — | — | 52 | 19 | 56·3 | 1·30 |
| | F | — | 1 | 3 | 1 | 6 | 1 | — | — | 12 | | | |
| (*f*) Archway Hospital | M | — | — | 5 | 20 | 29 | 16 | 1 | — | 71 | 14 | 63·1 | 0·94 |
| | F | — | — | 1 | 3 | 5 | 3 | — | — | 12 | | | |
| (*g*) St. Mary's Hospital, Islington | M | — | — | 2 | 20 | 38 | 16 | 2 | — | 78 | 21 | 64·3 | 0·83 |
| | F | — | 1 | 1 | 4 | 10 | 4 | 1 | — | 21 | | | |
| (*h*) Highgate Hospital | M | — | 1 | 2 | 8 | 18 | 11 | 4 | — | 44 | 15 | 66·9 | 1·53 |
| | F | — | — | — | — | 2 | 6 | — | — | 8 | | | |
| All hospitals | M | 1 | 16 | 72 | 186 | 242 | 82 | 11 | 1 | 611 | 17·5 | 60·4 | 0·40 |
| | F | — | 12 | 14 | 29 | 44 | 24 | 7 | — | 130 | | 60·8 | 1·12 |
| (*a*), (*c*), (*d*) and (*e*) together | M | 1 | 12 | 53 | 113 | 123 | 17 | 1 | 1 | 321 | 18·5 | 57·5 | 0·51 |
| | F | — | 10 | 12 | 19 | 21 | 8 | 3 | — | 73 | | 56·9 | 1·52 |
| (*b*), (*f*), (*g*) and (*h*) together | M | — | 4 | 19 | 73 | 119 | 65 | 10 | — | 290 | 16·4 | 63·7 | 0·63 |
| | F | — | 2 | 2 | 10 | 23 | 16 | 4 | — | 57 | | 65·7 | 1·39 |

Abbots to 22 per cent at the Middlesex Hospital, but the differences are not significant. The proportion of females in the whole series (17·5 per cent) compares well with 17·2 per cent, the proportion of females among all deaths from carcinoma of the lung in the Administrative County of London during the same period (Registrar General, 1950, 1951, 1952, 1953).

(ii) *Age.*—In this respect the hospitals are divisible into two distinct groups. In the first, the teaching hospital group, the average age of the patients at necropsy was less than 60 years (generally about 57 years). In the second, the Regional Board hospital group, the average age exceeded 60 years (generally 63 or 64 years). The mean age of all the patients was 60·5 years, but this figure alone is not of much importance in view of the above heterogeneity. Table II shows the age distribution of the patients for the various hospitals together with the mean ages and the standard errors of these mean values.

It is of interest to compare the age distributions for the two groups of hospitals referred to above with the age distribution for deaths in England and Wales during 1948–52 (Registrar General, 1950, 1951, 1952, 1953). This is done separately for males and females in Fig. 1. It will be seen from this that the two groups of hospitals show exactly opposite features in this respect. The teaching group with the low average age at death has an excess of young and

middle-aged people in both sexes, but a deficiency of people over seventy years. In the Regional Board group, with high average ages, there is a relatively lower proportion of persons under sixty.

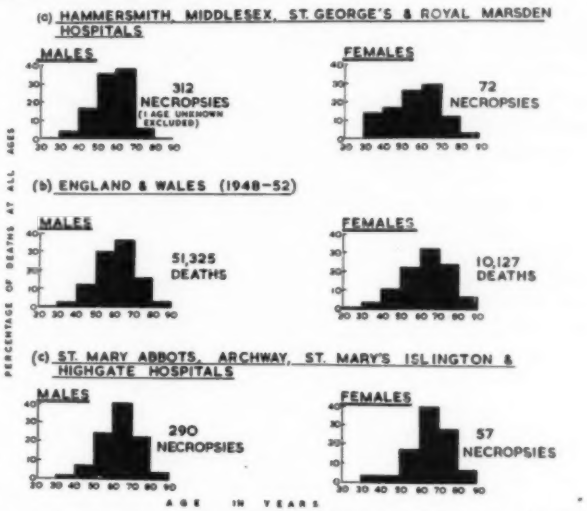


Fig. 1.—Proportionate distributions of ages at death compared for necropsies at two groups of hospitals and for all deaths from cancer of the lung in England and Wales.

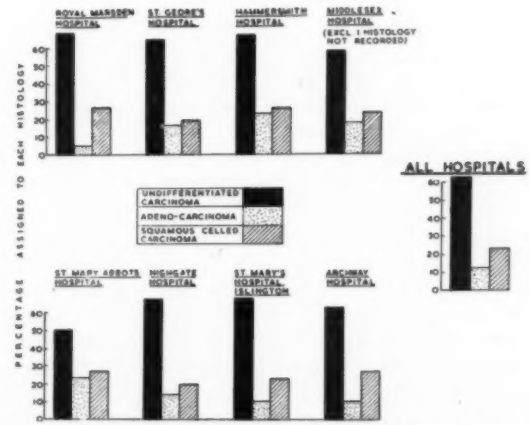


Fig. 2.—Proportionate distributions according to histology for each hospital.

(iii) *Histology*.—In considering histology we are faced with a new source of heterogeneity, namely, that arising from the individual interpretation of sections by different pathologists. Fig. 2 shows how the patients in the series were assigned to three main groups; undifferentiated carcinoma, adenocarcinoma and

squamous celled carcinoma. In the term " undifferentiated " we have included the following :

(*a*) " oat celled carcinoma ",
(*b*) " round celled carcinoma ",
(*c*) " polygonal celled carcinoma ",
(*d*) " anaplastic carcinoma " ;

while " adenocarcinoma " includes those reported both as " adenocarcinoma " and as " columnar celled carcinoma ".

It will be seen that the undifferentiated group is by far the largest, making up 63·5 per cent of the whole series. Adenocarcinomas and squamous celled carcinomas account for 12·6 per cent and 23·9 per cent respectively. With regard to differences between hospitals, we find that the proportion assigned to squamous celled carcinoma ranges from 19 per cent at Highgate Hospital to 27 per cent at the Royal Marsden Hospital, though these differences are not significant. This is not the case when we come to the question of assessing differentiation between undifferentiated tumours and adenocarcinomas. Here the differences are highly significant and are almost certainly related to lack of uniformity in interpretation rather than to processes of selection. Taking undifferentiated tumours and adenocarcinomas together we find that the proportion of these which are designated as adenocarcinomas varies from 6 per cent at the Royal Marsden Hospital to 31 per cent at St. Mary Abbots Hospital.

(iv) *Age and sex.*—It is not proposed to discuss here how the age distribution of the series varies in the two sexes from one hospital to another. We may, however, examine whether there are any differences between the two groups of hospitals referred to in (ii). The age distribution of these two groups according to sex is set out at the bottom of Table II.

A study of these data reveals the following features :

(*a*) In the teaching group there is a marked difference in the way in which male and female ages are distributed, although the average ages do not differ much. The ratio of males to females reaches a maximum at about sixty but becomes less towards the extremes.

(*b*) In the Regional Board group there is some indication that the average age of females is greater, though the differences in the manner of distribution of male and female ages is much less marked.

(v) *Sex and histology.*—In the present series, 89 per cent of all squamous celled carcinomas were in male patients, as against 80·5 per cent in males among other histological types. This supports the already well established view that squamous celled carcinoma of the lung is even more a disease of men than is cancer of the lung in general. The adenocarcinomas show a higher proportion of females, but the evidence that this is a real difference in incidence is not very strong. These features are illustrated graphically in Fig. 3.

(vi) *Age and histology.*—There is some indication that squamous celled carcinoma relative to other types attains a maximum incidence in the sixties. In similar comparisons between undifferentiated and differentiated types of tumour the situation is somewhat obscured by lack of uniformity in classification, though in spite of this there do not seem to be any real differences in relative frequency according to age. The variation in the relative incidence of squamous celled carcinoma is seen in Fig. 4.

*Incidence of metastases of all types*

Differences between hospitals in the selection of material for necropsy has presented serious difficulties, but those arising from variations in necropsy findings are even greater. So much seems to depend on pathological techniques, on amount of work in relation to availability of staff, and on the particular

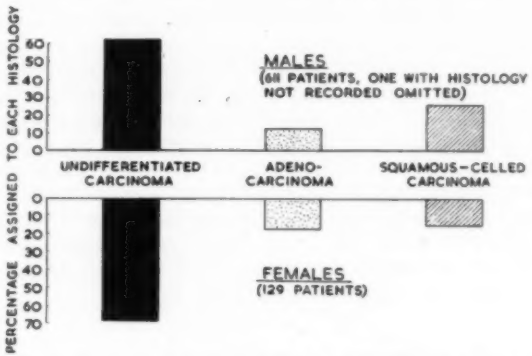


FIG. 3.—Proportionate distribution according to histology compared for males and females.

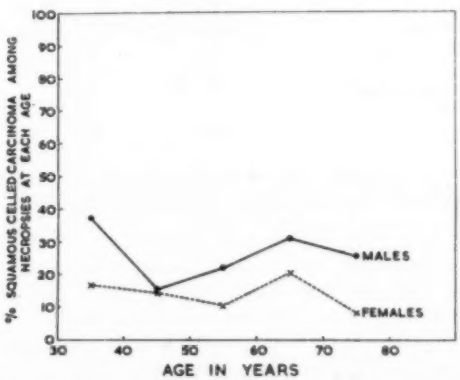


FIG. 4.—Relative incidence of squamous celled carcinoma found at necropsy for each decade of age.

interests of the pathologists reporting. We propose, therefore, in presenting the information collected from the records to suggest hypotheses rather than to attempt to draw definite inferences.

It has been remarked earlier that a small proportion of the patients had no intracranial examination at necropsy. Therefore in giving statistics relating to intracranial metastases these cases have been excluded from consideration. Indeed it might be argued that in considering the incidence of *all* metastases we should also exclude this group, since patients who did not have an intracranial examination and who had no observable metastases, might, in fact, have had

intracranial secondaries. We have, however, considered this (we hope correctly) to be unimportant and have calculated proportions without any such exclusions.

Of the 741 patients, 517 (69·8 per cent) were recorded as having blood-borne metastases at some site or other. The proportions for individual hospitals ranged from 60 per cent to 77 per cent.

(i) *Sex.*—There seems to be no difference in the tendencies for males and females to develop metastases. Secondaries were found in 69 per cent of males and 72 per cent of females.

(ii) *Histology.*—The data confirm the well established fact that squamous celled carcinoma exhibits much less tendency to metastasise than do other types. Metastases were found in association with squamous celled carcinoma at death in only 41 per cent of cases as compared with 79 per cent for other types. In the same way it is found that the adenocarcinomas have less tendency to metastasise than the undifferentiated tumours. Secondaries were found in 70 per cent of patients described as having adenocarcinomas, but in 80·4 per cent of patients said to have undifferentiated tumours. These percentages must, however, be accepted with caution in view of the evident differences in interpretation between hospitals. The figures are summarised in Table III.

TABLE III.—*The Proportions of Patients with Metastases at any Site as found at Necropsy according to Histology of Primary Tumour.*

| Histology. | Total number of patients. | Number with any metastasis. | Per cent with any metastasis. |
|---|---|---|---|
| Undifferentiated | 470 | 378 | 80·4 |
| Adenocarcinoma | 93 | 65 | 69·9 |
| Squamous celled carcinoma | 177 | 73 | 41·2 |
| Type not recorded | 1 | 1 | — |
| All types | 741 | 517 | 69·8 |

(iii) *Age.*—It would appear that the incidence of metastases in general decreases with age. There are, however, three other possible explanations which we must first consider, namely :

(*a*) That the proportion of squamous celled carcinomas increases with age.

(*b*) That older patients who are generally more frail may have a greater tendency to die before metastases become established.

(*c*) That there may be a tendency for less thorough necropsies to be performed on aged patients at some hospitals.

It seems that (*a*) may be discounted at least on the basis of the present data. There is evidence that the proportion of squamous celled tumours is highest in the sixties but none that there is a uniform increase beyond these ages. We cannot dispose of (*b*) so easily, since in the present data no record was made of the duration of the disease. Any further investigation on these lines should certainly take account of this additional factor. We are left with (*c*) and it is conceivable that this may explain the observed decrease in part at least. In examining the incidence of metastases by age for separate hospitals it is quite evident that the extent and nature of the decrease varies. The agreement between hospitals is fairly good at younger ages, but becomes bad at advanced ages. It would seem, therefore, that whether or not tumours in older patients exhibit less tendency to

metastasize, the position may be confused by differences between hospitals in their attitude towards the post-mortem examination of aged patients.

(iv) *Sex and histology.*—A consideration of the interaction between these two factors reveals nothing significant.

(v) *Sex and age.*—In Fig. 5*a*, we see how the incidence of metastases is related to age for males and females separately. It would appear from this that females have a lower metastasis rate at ages below 45 but that at ages over 45 the position is reversed. This, together with the fact that the rate for females attains a peak at about 45 years while for males it decreases continually, might suggest the influence of hormone balance on the development of metastases. If, however, we correct for the proportions of different histologies at each age the excess incidence for females largely vanishes, though the male excess at early ages is accentuated (Fig. 5*b*).

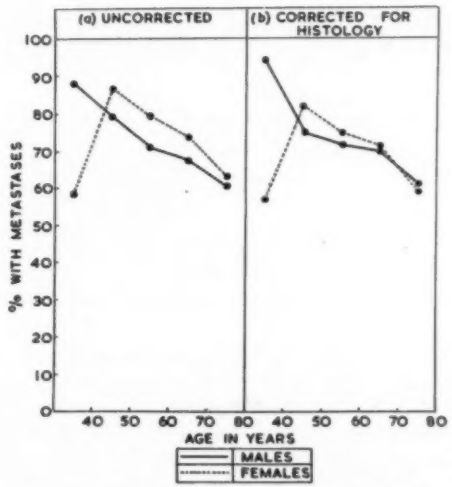


FIG. 5.—Incidence of metastases by sex and age.

(vi) *Age and histology.*—The decline in the general metastasis rate with age noted above appears to hold for individual histological types in varying degrees, although the position is confused for the reasons given in (iii) above. All the figures are, however, illustrated in Fig. 6.

From this point we shall adopt a similar scheme in examining the incidence of metastases at particular sites of the body. We have seen that undifferentiated tumours are much more likely to produce metastases than are squamous celled tumours. This may be due to the fact that undifferentiated emboli have a greater overall potentiality for growth than squamous cells. On the other hand it may mean that undifferentiated tumours liberate more emboli than do squamous-celled tumours, but that the emboli which are liberated in either case have a growth potential which, though not inherently very different, may differ according to the host tissues in which they lodge. Under the latter hypothesis it is of interest to consider the incidence of specific metastases from a certain type of

tumour expressed as a proportion of the total number of patients with tumours of that type *who were noted as having metastases*. It is proposed to express the incidence in this way whenever histological type is a factor under consideration.

*The incidence of metastases at individual sites.*

There is no doubt that the liver is the organ in which metastases from carcinoma of the bronchus are most commonly found. In this series 39·3 per cent of necropsies revealed secondaries in this organ. It is also worth noting that there was quite good agreement between the various hospitals; proportions observed ranged from 36 to 44 per cent, except for a figure of 27 per cent in one rather small group.

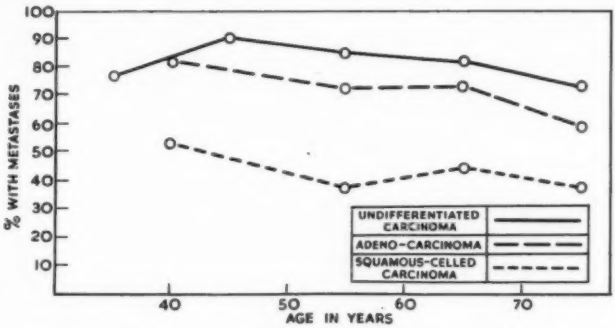


FIG. 6.—Incidence of metastases according to age and histology.

The only other site for which the incidence of metastases approaches that of the liver is the adrenals. The proportion observed with metastases in these organs is 33·5 per cent. In this case, however, the agreement between hospitals is not so good and extends from 25 to 47 per cent. When we consider the relative sizes of liver and adrenals, this high rate is seen to be a matter of outstanding interest.

Next in order of relative frequency are brain metastases. We are obliged to base the incidence at this site on those cases in which an intra-cranial examination was performed. The incidence in this group is 25·7 per cent, with figures for individual hospitals extending from 17 to 43 per cent. A special selection process operates at the latter hospital where there is considerable interest in neuro-surgery. It is indeed a remarkable fact that many patients attending this hospital on account of neurological symptoms alone are subsequently found at necropsy to have a primary tumour in the lung.

Metastases in kidneys were recorded in 15·4 per cent of necropsies with figures for individual hospitals ranging from 12 to 25 per cent.

The overall incidence of bone metastases in the series is 14·6 per cent, but this has little import when we observe that the incidence for the separate hospitals is widely dissimilar (6 per cent to 27 per cent) and general, detailed skeletal examination uncommon. It is clearly futile to attempt to draw any conclusions from data relating to bone metastases unless very detailed necropsies were performed with this object in view.

The only other organ in which metastases occur to an appreciable extent is the pancreas. In the whole series the pancreas was recorded as being invaded in 11·6 per cent of cases with a range of 7 to 27 per cent between the hospitals.

Other sites of some interest are the spleen and the thyroid, but the incidence of metastases recorded for these organs is rather small being 5·3 and 3·6 per cent respectively.

The only other organs which will subsequently be referred to specifically are the ovaries. Ovarian metastases were noted in seven patients, i.e. 5·4 per cent of females in the series.

Other sites for which metastases have been recorded include the pituitary (7), the prostate (3), the testis (1), the alimentary tract (37), the meninges (11), the heart (17), the medulla (1), the uterus (1), and skin (12). These will not be referred to again here either because of their rarity or because of an evident lack of uniformity in the frequency with which they are reported by different hospitals. Table IV gives the figures for the overall incidence of specific metastases together with the range of values provided by the data from the various hospitals.

TABLE IV.—*The Observed Incidence of Metastases at Various Sites*

| Site. | Number of metastases reported. | Overall incidence (%). | Range (%). |
|---|---|---|---|
| Liver | 291 | 39·3 | 27 44 |
| Adrenals | 247 | 33·5 | 25 47 |
| Brain* (excluding meninges) | 166 | 25·7 | 17 43 |
| Kidney | 114 | 15·4 | 12 25 |
| Bones | 108 | 14·6 | 8 27 |
| Pancreas | 86 | 11·6 | 7 27 |
| Spleen | 39 | 5·3 | 2 10 |
| Thyroid | 27 | 3·6 | 1 11 |
| Ovary† | 7 | 5·4 | |

* Percentage of necropsies at which an intracranial examination was made.
† Percentage of females only.

(i) *Sex.*—The two sexes differ in only one remarkable way in regard to the incidence of metastases at individual sites. This may be due to the fact that in basing metastasis rates on the whole data we are missing important features. We shall, however, examine the effect of age and histology jointly with sex in subsequent sections. At most sites there is a slightly higher rate of metastasis for females. This may be due in part at least to the less frequent occurrence of squamous celled tumours among females.

The adrenals form the one exception, since here the metastasis rate for males is higher than for females, and although this difference is not significant for the whole data taken together it does, as we shall see, assume greater importance when age is also considered. The percentage incidence for metastases is shown by site for males and females in Table V.

(ii) *Histology.*—The same incidence gradient from undifferentiated to squamous is seen for metastases at particular sites as for all metastases taken together. The gradient is, however, considerably modified in the case of secondaries in the kidney. It is, perhaps, more instructive at this stage to consider incidence based not on all necropsies but only on those revealing metastases at any site. We

shall then be answering the question : " given that metastases have occurred, is there any difference in the manner in which they are distributed through the body for primary tumours of different histology ? "

TABLE V.—*The Incidence of Metastases at Various Sites according to Sex*

| Site. | Males. | | Females. | |
|---|---|---|---|---|
| | Number with metastases. | Per cent. | Number with metastases. | Per cent. |
| Liver | 235 | 38·5 | 56 | 43·1 |
| Adrenals | 212 | 34·7 | 36 | 27·7 |
| Brain* (excluding meninges) | 138 | 25·7 | 28 | 25·5 |
| Kidneys | 90 | 14·7 | 24 | 18·5 |
| Bones | 90 | 14·7 | 18 | 13·8 |
| Pancreas | 66 | 10·8 | 20 | 15·4 |
| Spleen | 28 | 4·6 | 11 | 8·5 |
| Thyroid | 21 | 3·4 | 6 | 4·6 |
| All organs | 424 | 69·4 | 93 | 71·5 |

*Based on 537 males and 110 females who were examined intracranially at necropsy.

When we examine these modified metastasis rates we find that there is much more agreement between different histologies for metastases at most sites, e.g. liver, adrenal, spleen, thyroid and bones. This procedure does, however, emphasize the differences for kidney and pancreas. In the case of kidney the rate for undifferentiated tumours is relatively low, while for pancreas the corresponding rate is still rather greater than for the other types. These features are illustrated in Fig. 7.

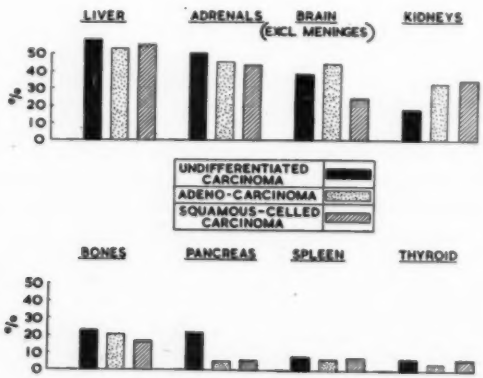


FIG. 7.—Proportions of patients with metastases who had metastases in certain specified organs.

(iii) *Age.*—The tendency for metastases to become less common with advancing age is, in general, preserved for metastases in individual organs, though there is usually some modification in the overall pattern We are also at the mercy of the difficulties referred to under the heading Incidence of Metastases of all Types, Section (iii).

The incidence of liver metastases seems to remain fairly constant at about 39 per cent throughout the age range, though there is a possibility that it is higher in the forties (48 per cent).

The position relating to brain secondaries is not very clear. The overall incidence is found to be 25·7 per cent, but the variation about this figure is considerable at individual hospitals. Furthermore, the variation of incidence with age does not follow a consistent trend at different hospitals. The data of two hospitals point to a fairly level incidence of about 30 per cent, while at another the picture is biased by neurosurgical interests. The remainder indicates an incidence declining from 46 per cent at ages under 50, to 12 per cent in old age. Brain metastases are dealt with further under the heading Brain Metastases from Bronchial Carcinoma.

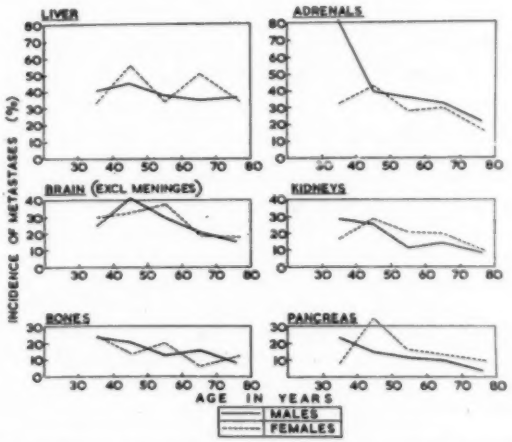


FIG. 8.—The incidence of metastases at various sites according to age and sex.

Adrenal metastases occur very commonly at ages under 40 years (61 per cent) but see (v) below.

In regard to ovarian metastases it seems important that five out of the seven instances recorded occurred in patients less than fifty years of age.

(iv) *Sex and histology.*—Nothing significant emerges from a consideration of these two factors jointly.

(v) *Sex and age.*—The high incidence of adrenal metastases in patients dying at an early age was found to be particularly characteristic of males. Fourteen out of 17 (82 per cent) necropsies performed on males under 40 years of age revealed adrenal metastases. Fig. 8 shows in detail the incidence of metastases at various sites according to sex and age.

(vi) *Histology and age.*—Here again we treat incidence as based on those patients in whom some metastasis was found, i.e. relative incidence. This analysis brings out once more the higher relative incidence of kidney metastases from adenocarcinomas and squamous celled tumours as compared with undifferentiated tumours, particularly at ages over 50.

(vii) *Mechanical factors.*—It is of interest to see whether the incidence of metastases in particular organs is related in any way to such basic factors as weight of tissue, total blood flow through the organ in unit time and total blood flow in unit time per gramme of tissue. The relevant figures are set out in Table VI. It will be seen that if there is any relationship it must be a very complex one, doubtless involving other variables. There might conceivably be some direct dependence on the weight of the organ, since both liver and brain show such high incidence. This alone would leave unexplained, however, the frequent occurrence of adrenal secondaries, not to mention the infrequent occurrence of secondaries in such an extensive organ as the skin, although the post-mortem fading of these secondaries is well recognised.

Any suggestion that the incidence of metastases might be inversely related to the velocity of blood flow is readily countered by the example of the spleen through which the blood flows much more slowly than through the brain. Furthermore, the extent of the capillary bed does not seem to exert a marked influence, since the skin and skeletal muscles are rarely affected.

TABLE VI.—*Frequency of Metastases from Carcinoma of the Bronchus Related to Weight of and Blood Flow in Various Organs*

| Organ. | Percentage of all patients having metastases in organ. | Weight of organ in grammes.* | Blood flow per 100 g. of tissue in c.c. per minute.† | Approximate blood flow in the whole organ in c.c. per minute. |
|---|---|---|---|---|
| Adrenals | 33·5 | 12 | 600–700 | 80 |
| Thyroid | 3·6 | 40 | 560 | 220 |
| Pancreas | 11·6 | 110 | — | — |
| Spleen | 5·3 | 155 | 40 | 60 |
| Kidneys | 15·4 | 300 | 400 | 1300 |
| Brain | 25·7 | M 1400<br>F 1275 | 200 | 2800 |
| Liver | 39·3 | 1650 | 50 (arterial) | 800 |

* Sunderman and Boerner (1950).
† Best and Taylor (1950).

Since the elementary haemodynamic processes seem to have no simple bearing on the distribution of metastases from carcinoma of the bronchus some more subtle differences in capillary bed structure or in by-passing mechanisms must be invoked if the mechanical theories are to be upheld. Detailed work on this aspect of the problem in man is required and might perhaps come from the use of micro-radiographic techniques similar to those employed by Barclay (1951).

The " seed and soil " aspect of this problem is likely to be concerned with an interaction between the structure and biochemical composition of the host tissues, on the one hand, and the histological characteristics of the emboli, on the other.

*Brain metastases from bronchial carcinoma*

This subject has aroused much interest for some years and for this reason the brain metastases recorded in connection with the present data are being made the subject of a separate article.

The proportion of necropsies revealing brain metastases, namely 25·7 per cent, appears to be consistent with the results of similar investigations over the past 40 years.

It is shown that brain metastases tend to be associated with metastases in other organs but that the extent of this association varies for different organs, e.g. the association with liver metastases is low but high with metastases in the pancreas. It is suggested that these differences may arise merely from relative lethality.

It is also shown that brain metastases associated with metastases elsewhere in the body have a greater tendency to be multifocal.

The proportion of single brain metastases found to be located in the cerebellum (38 per cent) does not in itself suggest any more interesting hypothesis than a direct relationship to tissue mass.

### *Conclusions*

(i) A study of the incidence of metastases in selected organs in relation to the haemodynamic normal values associated with them reveals no simple relationship though there does appear to be some correlation between the occurrence of metastases and the weights of organs, with the adrenals as the outstanding exception.

(ii) The high incidence of adrenal metastases in young males, together with the fact that the few instances of ovarian metastases were found mainly at ages under 50, might suggest a relationship between metastasis rate and biological activity in these endocrine organs at least.

(iii) The proportion of adenocarcinomas classified as undifferentiated tumours of the bronchus varies greatly among different pathologists. The proportion of undifferentiated tumours and adenocarcinomas taken together which were classified as adenocarcinomas varied from 6 to 31 per cent in this series. Particularly careful and critical examination is therefore required in drawing conclusions regarding different aetiologies in groups with different histologies.

(iv) It would seem likely that the incidence of metastases and their distribution depends on a complicated balance between the following factors : degree of tumour cell differentiation, state of the tissues of the tumour bed, number of cells liberated, size of adhering clumps, size of organs, rate of blood flow at different sites, extent and nature of capillary beds and the metabolic activity of different tissues.

There is still too little data available on many of these points. There are however, enough exceptions (for example adrenals to dependence on organ size, kidneys to dependence on degree of tumour cell differentiation, spleen to dependence on rate of blood flow) to many of the more simple factors to make it highly improbable that any purely mechanical theory could alone be held responsible for metastases. A detailed study of the more obvious exceptions to the simple theories might provide useful information about the factors determining the incidence and distribution of blood-borne metastases, that is to say the main factors which are at present delaying further advance in the effectiveness of cancer treatment.

### SUMMARY

1. The records of 741 necropsies relating to carcinoma of the bronchus drawn from eight London Hospitals (four teaching and four Regional Board hospitals) have been analysed.

2. These necropsies were taken from a total of 10,430 performed at these hospitals in the years 1948–52. In the hospitals which do not specialize in malignant disease the proportion of all necropsies relating to lung cancer approaches 7 per cent.

3. The proportion of females in this material was 17·5 per cent, corresponding to a ratio of 4·7 males to 1 female.

4. The mean age at death of the patients was about 57 years in the Teaching Hospitals and about 64 years in the Regional Board Hospitals.

5. The greater proportion of necropsies, 63·5 per cent, revealed undifferentiated carcinoma ; 23·9 per cent revealed squamous celled carcinoma and 12·6 per cent adenocarcinoma. Differences of interpretation between hospitals may, however, affect the validity of these figures, particularly the last.

6. There was a somewhat higher proportion of squamous celled carcinoma among males.

7. In 69·8 per cent of cases metastases were found at some site or other. For individual histological types the corresponding figures are : undifferentiated carcinoma 80·4 per cent, adenocarcinoma 70 per cent, squamous celled carcinoma 41 per cent.

8. An apparent tendency was found for the metastasis rate to decline with age, probably due, in part, to some differences in attitude towards the post-mortem examination of aged patients.

9. The most common sites for metastasis were found to be liver (39·3 per cent), adrenals (33·5 per cent) and brain (25·7 per cent), though only in the case of the liver was there good agreement between hospitals.

10. The only marked sex-difference in the incidence of metastases was found in the higher incidence of adrenal metastases among males dying at ages under 40 years. Out of seventeen such cases, the adrenals were found to be involved in fourteen.

We are especially grateful to a number of pathologists who most generously have allowed us free access to their necropsy records and permission to publish the results of our study of them : they were Professor T. Crawford of St. George's Hospital, Professor J. H. Dibble of Hammersmith Hospital, Professor R. W. Scarff of Middlesex Hospital, Dr. A. B. Bratton and Dr. C. C. Bryson of the Whittington Hospital, Dr. A. G. Signy and Dr. M. Gillespie of St. Mary Abbots Hospital and Dr. J. W. Whittick of the Royal Marsden Hospital.

We are indebted to Professor D. W. Smithers who both suggested this investigation to us and has given us much valuable assistance in the preparation of the text.

While acknowledging this help and kindness we must make it clear that they cannot be held in any way responsible for the comments we have made on the data collected.

*Riassunto :* La distribuzione e la crescita delle metastasi ematiche dei tumori appare, secondo le odierne conoscenze, dipendere da una complessa associazione tra fattori meccanici e biochimici.

Poichè il carcinoma bronchiale dell'uomo offre, per la stessa sua sede, il miglior materiale di studio del problema, gli AA. hanno scelto questo tipo di

tumore e ne hanno studiato la distribuzione delle metastasi ematiche in 741 casi tratti da 10·430 autopsie eseguite in 8 Ospedali di Londra dal 1948 al 1952.

Le principali osservazioni derivanti sono: (a) la conferma che i vari tipi istologici esibiscono una diversa attitudine a metastatizzare; (b) che la tendenza alla diffusione metastatica diminuisce con l'aumentare dell'età; (c) che vi è una tendenza a metastasi associate tra certi organi; (d) che la distribuzione delle metastasi nei vari organi si dimostra soggetta ad una interazione tra semplici processi emodinamici, caratteristiche istologiche degli emboli neoplastici e biochimismo del tessuto ospitante.

La elevata incidenza di metastasi surrenali nei maschi di età relativamente giovane insieme al fatto che gran parte dei pochi esempi di metastasi ovariche nelle donne sono state trovate ad età inferiori ai 50 anni, può suggerire una relazione tra possibilità di impianto delle metastasi e attività biologica di questi organi.

## REFERENCES


ANDERSON, R.—(1951) *J. Neurosurg.*, **8**, 411.

BARCLAY, A. E.—(1951) 'Micro-arteriography; and other Radiological Techniques Employed in Biological Research.' Oxford (Blackwell Scientific Publications).

BATSON, O. V.—(1940) *Ann. Surg.*, **112**, 138.—(1942) *Amer. J. Roentgenol.*, **48**, 715.

BEST, C. H. AND TAYLOR, N. B.—(1950) 'The Physiological Basis of Medical Practice.' 5th Ed. London (Ballière, Tindall and Cox).

BRITISH EMPIRE CANCER CAMPAIGN.—(1950) *Ann. Rep.* **28**, 97.

COMAN, D. R., DE LONG, R. P. AND MCCUTCHEON, M.—(1951) *Cancer Res.*, **11**, 648.

DE LONG, R. P. AND COMAN, D. R.—(1950) *Ibid.*, **10**, 513.

HEADY, J. A. AND KENNAWAY, E. L.—(1949) *Brit. J. Cancer*, **3**, 311.

KREYBERG, L. AND POPPE, E.—(1940) *Lancet*, i, 593.

LUCKE, B., BREEDIS, C., WOO, Z. P., BERWICK, L. AND NOWELL, P.—(1952) *Cancer, Res.*, **12**, 734.

REGISTRAR-GENERAL (1950, 1951, 1952, 1953) *Statistical Reviews of England and Wales for the Years* 1948 *to* 1952. Tables Part I. Medical. London (H.M. Stationery Office).

STEINER, P. E., BUTT, E. M. AND EDMONDSON, H. A.—(1950) *J. nat. Cancer Inst.*, **11**, 497.

SUGARBAKER, E. D.—(1952) *Cancer*, **5**, 606.

SUNDERMAN, F. W. AND BOERNER, F.—(1950) 'Normal Values in Clinical Medicine.' Philadelphia and London (W. B. Saunders Company).

TRUETA, J., BARCLAY, A. E., DANIEL, P. M., FRANKLIN, K. J. AND PRITCHARD, M. M. L.—(1947) 'Studies of the Renal Circulation', p. 127, Footnote 1. Oxford (Blackwell Scientific Publications).

WATANABE, S.—(1954) *Cancer*, **7**, 215.

WILLIS, R. A.—(1952) 'The Spread of Tumours in the Human Body.' 2nd Ed. London (Butterworth).

YOUNG, J. S. AND GRIFFITH, H. D.—(1950) *J. Path. Bact.*, **62**, 293.

ZEIDMAN, I. AND BUSS, J. M.—(1952) *Cancer Res.*, **12**, 731.

*Idem*, MCCUTCHEON, M. AND COMAN, D. R.—(1950), *Ibid.*, **10**, 357.

# THE AETIOLOGY OF RESPIRATORY TRACT CANCER IN THE SOUTH AFRICAN BANTU

## PART I. CLINICAL ASPECTS

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## PART II. CHEMICAL IDENTIFICATION OF CARCINOGENS

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## PART I.

THE human respiratory tract is subject to a wide range of suspended irritants arising from atmospheric pollution, industrial dusts and fumes, or personal habits. Since these factors differ markedly in various communities, it is not surprising that the relative frequencies of cancer in the various respiratory organs of the African is very different from that obtaining in the European. During the six-year period 1949 to 1954, 86 cases of respiratory tract cancer in African patients were referred to the Radiation Therapy Department of this hospital, making up about 7 per cent of all malignant tumours seen, a proportion similar to that found in Europeans. These cases were drawn from a population of about 2,000,000 Transvaal Bantu of whom about one-quarter are urbanised and resident on the Witwatersrand. In this environment, exposure to the carcinogenic respiratory irritants commonly encountered in Europe is comparatively infrequent.

Probably no more than one-quarter of cancers occurring in this population actually reach hospital, so that the true incidence of the disease will be greater than our figures suggest. On the other hand, conclusions drawn from the relative frequencies of tumours in the different anatomical sites of the respiratory system are probably valid, particularly as there are, in this matter, very considerable racial differences.

### *Anatomical distribution*

For the purpose of this analysis, the respiratory tract has been divided into four zones : nose and accessory sinuses, nasopharynx, larynx and lungs. The relative frequency of involvement of each of these sites is shown in Table I, together with comparative data from Europeans (Shapiro, Keen, Cohen and de Moor, 1955).

In both sexes the incidence of lung cancer is relatively high and tumours of the nasal region are comparatively rare in the European, while in the African there is a high incidence of carcinoma of the nose and sinuses, and lung cancer is relatively infrequent.

TABLE I.—*Relative Frequencies of Cancer of Respiratory Tract by Sex and Site*

Radiation Therapy Department, Johannesburg General Hospital, 1949–1954

| Anatomical site. | Bantu patients. (Present series.) Males. | Females. | Total. | Per cent. | Europeans. (Shapiro, *et al.*, 1955.) Males. | Females. | Total. | Per cent. |
|---|---|---|---|---|---|---|---|---|
| Nose and accessory sinuses | 38 | 8 | 46 | 54 | 4 | 3 | 7 | 5 |
| Naso-pharynx | 7 | 5 | 12 | 14 | 10 | 4 | 14 | 10 |
| Larynx | 13 | 0 | 13 | 15 | 23 | 2 | 25 | 18 |
| Lungs | 15 | 0 | 15 | 17 | 80 | 14 | 94 | 67 |
| Totals | 73 | 13 | 86 | 100 | 117 | 23 | 140 | 100 |

Neoplasms of the *nose and accessory sinuses* account for more than one-half of respiratory tract malignancy in the Bantu but only 5 per cent of the corresponding group in Europeans. The region of the maxillary antrum seemed to be the most frequent site of origin, 39 of the 46 cases having apparently arisen in this area, although at the time of examination the disease was usually so advanced, with ulceration of the palate and invasion of the orbit, nasal cavity, and ethmoid region, that the exact site of origin was obscured. Fig. 1 and 2 show typical examples of cases when first seen, and Öhngren's (1933) classification was obviously inapplicable. Six cases seemed to have started in the vicinity of the ethmoid cells, and one was thought to have originated in the sphenoidal sinus.

In all but 6 of the foregoing patients a definite histological report was obtained, and of these 83 per cent (33 cases) were well differentiated squamous-cell carcinomata, one patient had an adenocarcinoma, 3 were diagnosed as " transitional " and " Schneiderian " carcinomata, and 3 were sarcomata. Of the latter, one was radio-resistant and presumably of connective tissue origin, the remaining two were extremely radio-sensitive lesions presumably of reticulo-endothelial origin (Figs. 3 and 4). In all cases the primary growth was very extensive, but in spite of the advanced stage of the disease, lymphatic spread and distant metastases were very infrequent and late manifestations. Only 7 cases were considered too advanced for any radical treatment when first seen ; the remainder being treated with intensive radiotherapy, although the probability of cure was inevitably small.

Of the 12 *nasopharyngeal tumours*, 3 were squamous cell carcinomata, 2 lympho-epitheliomata, and 7 were diagnosed as spheroidal celled or anaplastic carcinomata. The ratio of 5 females to 7 males suggests that the preponderance of males is somewhat less than in other respiratory cancers, though the difference is not statistically significant. In only 2 cases was the disease localised to the primary site, the remainder having cervical lymph-node involvement, while 2 patients developed distant metastases.

*Carcinoma of the larynx* showed no significant racial predisposition, and all cases occurred in males, emphasising the well-known male proponderance of this disease. Of the 13 cases, 11 were squamous carcinomata and 2 were anaplastic tumours. Two patients had " early " or " intrinsic " tumours, 4 were locally advanced, 5 had regional lymph-node involvement, and 2 had distant metastases.

The 15 cases of *lung cancer*, representing 17 per cent of the series, shows the relatively low incidence compared with Europeans. All were males and all were

cigarette smokers. Twelve patients had squamous carcinomata and three had anaplastic tumours. In only one African with lung cancer was the disease reasonably early, 9 were locally advanced, 3 with mediastinal lymphadenopathy and 2 had distant metastases.

*Comparative incidence*

The frequency of cancer of the respiratory tract compared with the total number of malignant tumours seen in the African is approximately equal to that in the European. However, the foregoing data indicate that the incidence of carcinoma of the nose and accessory sinuses is exceptionally high in Transvaal Bantu. They also suggest that carcinoma of the lung is less frequent than in the European section of the community, while tumours of the pharynx and larynx seem to be equally frequent in the two races. The reliability of these conclusions is limited by the fact that the patients were selected specifically for one form of treatment (radiotherapy), and the process of selection may have differed in the two racial groups. A bronchogenic carcinoma in a rural African is more likely to be misdiagnosed than in the case of an urban European, the symptoms being ascribed, perhaps, to pulmonary tuberculosis, which is particularly common in the former group. The frequency of lung cancer in the Bantu, apparently only one quarter of that in the European (Table I), is probably underestimated, although the true incidence is almost certainly much less than in the European.

The relative preponderance of nasal and antral cancer in the Transvaal Bantu, on the other hand, is statistically unequivocal. Irrespective of the selection procedure, the figures can lead to no conclusion other than that the true incidence is several times greater in the Bantu than in the European. Cancer of the nose and paranasal sinuses accounts for fully 4 per cent of all tumours in Africans referred for therapy, but only 0·4 per cent of our European radiotherapy cases. A more accurate comparison is given by the Danish Cancer Registry (Clemmesen, 1951) where, of 2222 cancers of the respiratory system (4·8 per cent of all malignancy) only 130 (0·3 per cent of all tumours) affected the nasal cavity and accessory sinuses, a proportion similar to our European data. The figures seem to indicate, therefore, that the true incidence of cancer of the upper respiratory tract in the Bantu is about ten times greater than in the European. Further, unlike skin cancer in which the unusual features observed in the Bantu are shared by all pigmented peoples (Shapiro, Keen, Cohen and Murray, 1953), and cancer of the liver which is common to both primitive African and Eastern communities (Berman, 1951), the incidence of nasal and antral tumours in other pigmented peoples, including some relatively primitive communities, is not significantly greater than in the European (Khanolkar, 1951 ; Kouwenaar, 1951). In this respect the exceptionally high incidence of cancer involving the nose and paranasal sinuses seems

EXPLANATION OF PLATES

FIG. 1.—Typical appearance of Bantu patient with carcinoma of the maxillary antrum ulcerating the skin and extending into the palate, nasal cavity and orbit.

FIG. 2.—Carcinoma of the roof of the nasal cavity affecting the ethmoid region.

FIG. 3.—Typical advanced paranasal cancer immediately after insertion of a central radium source.

FIG. 4.—Same patient as in Fig. 3 one month after insertion of radium, showing a satisfactory palliative response.

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Keen, de Moor, Shapiro and Cohen.

to be a unique feature characteristic of the South African Bantu, and presumably dependent on local conditions.

*Aetiological factors*

A marked difference in tumour incidence of one racial group compared to another residing in the same geographical region, suggests the existence of either a hereditary predisposition or environmental aetiological factors. In this instance one might compare the age and sex distributions, dietary factors, atmospheric pollution, personal and tribal habits, and finally genetic factors, as affecting the two groups.

While the exact age distribution of the African population is not accurately known, a tentative survey (Eberhardt, 1949) suggested that it resembled that of the European population (1946 census) in the first five decades, but falls off more rapidly thereafter, thus giving a somewhat younger average age. Correspondingly, the average age of this series of cases (52 years) is lower than that reported in a similar series in Europeans (59 years, Shapiro, *et al.*, 1955), but there is no marked difference in age-susceptibility between nasal and bronchial cancer to account for the large racial differences found. The age distribution by decades of all African respiratory cancers referred to is shown in Table II, together with the standard population sample and the European age distribution. Similarly, the sex ratio, 85 per cent males in the whole series, is similar to that found in the corresponding tumours in Europeans, and suggests a constant predisposing endocrine effect on the respiratory mucosa independent of racial factors.

TABLE II.—*Age Distribution of Respiratory Tract Cancer in the Bantu*

| Age (years). | Nose. | Pharynx. | Larynx. | Lungs. | Total. | Population Census. (Bantu.) % | Population Census. (European.) % |
|---|---|---|---|---|---|---|---|
| 0– 9 | 1 | 0 | 0 | 0 | 1 | 24 | 22 |
| 10–19 | 1 | 1 | 0 | 0 | 2 | 18 | 18 |
| 20–29 | 2 | 0 | 0 | 0 | 2 | 17 | 16 |
| 30–39 | 5 | 3 | 1 | 2 | 11 | 16 | 16 |
| 40–49 | 12 | 3 | 3 | 4 | 22 | 14 | 11 |
| 50–59 | 10 | 3 | 3 | 6 | 22 | 5 | 8 |
| 60–69 | 8 | 1 | 4 | 2 | | | |
| 70–79 | 6 | 1 | 1 | 1 | 26 | 5 | 8 |
| 80–89 | 1 | 0 | 1 | 0 | | | |
| Total | 46 | 12 | 13 | 15 | 86 | 99 | 99 |
| Average age | 51 | 46 | 57 | 53 | 52 | | |

*Dietary deficiencies* have been suspected in many diseases showing racial differences, particularly in cancer of the liver. Diseases attributable to malnutrition, however, are common to large groups of African and Asian peoples subsisting on inadequate diets, while the preponderance of upper respiratory cancer appears to be unique in this country. Dietary factors, therefore, are probably not of importance in the aetiology of this disease.

*Pollution of the atmosphere* with carcinogens from domestic and industrial fuels is distinctly less marked on the Witwatersrand than in many urban centres in Europe. On the other hand, the whole of this area is exposed to dust residues from the gold-mining industry, consisting of silica and containing cyanide and

possibly uranium. However, all sections of the community are approximately equally exposed to these influences, so that the marked racial preponderance of nasal and antral cancer cannot be ascribed to atmospheric carcinogens.

*Personal habits*, insofar as they affect the respiratory tract, are of considerable interest. The statistical studies of Doll (1951) on the relation of smoking to lung cancer, the induction of tumours in mice painted with cigarette-smoke condensates (Wynder, Graham and Croniger, 1953), the identification of carcinogenic hydrocarbons in the smoke (Cooper and Lindsey, 1955), and the effect of particle-size on the absorption of smoke in different anatomical levels (Blacklock, Kennaway, Lewis and Urquhart, 1954), all form an impressive chain of evidence linking a personal habit with malignancy in one part of the respiratory tract. While smoking is widespread in the European, all of our series of 97 cases of bronchogenic squamous cell cancer being heavy smokers, the consumption of cigarettes by the Africans is a recently acquired habit and often limited by economic considerations. This factor could explain the lower incidence of lung cancer in the Bantu group. On the other hand, the Bantu tribes inhabiting the Transvaal and Natal provinces use extensively a form of snuff prepared from indigenous plants (Watt and Breyer-Bandwyk, 1932).

In view of the possible relationship between carcinoma of the paranasal sinuses and snuff-taking, an attempt was made to investigate this habit. Although accurate figures on our patients' permanent domiciles could not always be obtained, it was remarkable that very few, if any, of this series of cases were residents of the urban areas, most patients coming from the " native reserves ", primitive peasant communities subject to tribal authority and tradition.

In Higginson's (1951) survey of an urban Bantu group there was no obvious localisation of cancer in the upper respiratory tract, confirming our view that the high incidence of paranasal cancer is largely a rural phenomenon. Accordingly, information was obtained from missionaries, magistrates, district officers, and numerous other tribal authorities.

It appears that snuff-taking is, in most tribes, a universal habit of the older generation, and has been used from " time immemorial ". In certain tribes snuff-taking plays an important rôle in all tribal ceremonies and customs, including ancestor worship, rain-making ceremonies, and native medical practices (" bone throwing "). Consequently the habit usually commences when youths first participate in tribal and family ritual, that is in the early twenties, which is approximately 30 years prior to the average age of onset of this series of tumours. It is our impression, though we have no accurate figures, that snuff-taking by Bantu women is quite as common as amongst the men : women are certainly not excluded from the tribal ceremonies at which the snuff is used.

Tobacco is never the sole constituent of these snuffs, as it is considered insufficiently potent, and admixture of various incinerated plants or herbs is favoured. There are at least twenty different plants in common use, but the consistent favourite and the most potent is the aloe. Several species of aloe have been employed, but the one used in common in all tribes, when obtainable, is the *Aloe marlothii*, and this is the species we have had chemically investigated. Among those less commonly used which have been identified, are *Amarantus spinosus*, L., and some species of Senecio, Rhus, Mesembryanthemum and Arthemisia.

The dried plants are ignited in an open container, the resultant greyish ash is subsequently ground with stones, and small quantities of water added until the

mass becomes a dark, slightly oily, coarse-grained powder. Occasionally eucalyptus oil, lemon juice, and other aromatic herbs are added as flavouring agents. Approximately two parts of tobacco powder and one part of the plant ash mixture are then ground together but the relative proportions may be varied according to taste. A well-prepared snuff is extremely irritant to the nasal mucosa, and a period of initiation with milder mixtures is essential before the habit can be fully acquired. The amount used varies considerably, but the average daily ration is reported to be " as much as can be heaped on a sixpence ", though most of our paranasal cancer cases admitted to using more than twice that amount.

Examination of the aloe snuff showed the presence of nicotine and a series of polycyclic hydrocarbons, including the well-known carcinogen 3 : 4-benzpyrene, as described in the section following. Since it is known that a large proportion of particles greater than one or two microns in diameter are arrested in the upper respiratory tract and rarely reach the lung, it is to be expected that this coarse-grained material would, on inhalation, be almost entirely concentrated in the nasal region and thus might explain the anatomical distribution of the disease.

## Part II

In a previous communication (Campbell and Cooper, 1955) the identification in samples of snuff of certain polycyclic hydrocarbons was described. Since then examination of the constituents of the snuff has been carried out and the work is described here in full.

### *Bantu snuff*

As mentioned above, the snuffs are prepared from tobacco leaves and an " ash " made by burning the stems of certain plants : we have examined samples of Zulu and Venda snuffs made from the aloe and amarantus species respectively. We have also examined samples of sun-dried tobacco leaves and aloe stems, and of ashes, made from " aloe stems of Zulu origin " and from the wild plant " called by the Shangaan people Shi Tehava Misisi ".

### *Method of analysis*

The method is similar to that used for the examination of tobacco smoke condensate (Commins, Cooper and Lindsey, 1954). A weighed sample was extracted in a Soxhlet apparatus until, after about six hours, no more coloured or fluorescent material was extracted. In earlier experiments acetone was used as a solvent, but later samples were dried first for ten days in a desiccator over concentrated sulphuric acid and then extracted with chloroform. When the extraction was complete, the solvent was evaporated cautiously and the residue washed several times with hot cyclohexane to dissolve the polycyclic hydrocarbons : finally the washings were combined, and evaporated to about 5 ml. and chromatographed on alumina.

For the initial separation a column of alumina 2·5 cm. × 10 cm. was used. The material was eluted with cyclohexane gradually increasing the pressure to about a 10 cm. head of cyclohexane, at which point benzene was added in stages, 5, 10, 25, 50 to 100 per cent, and finally the column was washed with chloroform. The progress of the fluorescent bands was followed down the column with a U.V.

lamp, and these bands were eluted and collected in fractions of about 3 ml. Each fraction was examined for specific polycyclic hydrocarbons, using a Unicam S.P.500 spectrophotometer, and the compounds were identified by searching for peaks (Wedgwood and Cooper, 1953). The chromatographic order of the compounds was known from a previous examination of artificial mixtures. Fractions containing the identified hydrocarbons were combined, evaporated, taken up in cyclohexane and re-chromatographed on a column of alumina, 1 cm. × 3 cm., or if the background absorption remained high, a silica gel column of similar length was used. This procedure was repeated until the separation from background was good, when fractions were re-combined and the compounds determined by measuring the heights of peaks characteristic of each compound (Cooper, 1954 ; Fig. 5–12).

TABLE III.—*Comparative Composition of Snuff, Aloe Stems, Tobacco and Ash (parts per million)*

| | Zulu snuff. | | Venda snuff. | | Aloe stems. | | Tobacco. | | Aloe ash. | | Ash from Shi Tehava Misisi. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2* | 1 | 2* | 1 | 2 | |
| Anthracene | 0·05 | 0·07 | — | 0·01 | Present | 0·002 | 0·01 | 0·02 | 0·003 | — | 0·007 |
| Pyrene | 0·56 | 0·58 | 0·08 | 0·09 | 0·017 | 0·024 | 0·03 | 0·049 | 0·003 | 0·0015 | 0·020 |
| Fluoranthene | 0·80 | — | 0·12 | 0·15 | 0·034 | 0·085 | 0·110 | 0·108 | 0·006 | 0·006 | 0·018 |
| 3:4-Benzpyrene | 0·27 | 0·25 | Present | Present | Present | 0·004 | 0·006 | 0·010 | 0·002 | 0·002 | 0·013 |
| 1:12-Benzperylene | 0·14 | — | — | — | — | — | — | — | — | — | — |

* This material was dry, and hence gave some higher values.

## DISCUSSION

In early experiments acetone was used as solvent, but this extracted large quantities of unwanted aqueous gums and other coloured material which made the identification and isolation of the hydrocarbons difficult and indeed may have absorbed some of the material. Extraction with chloroform after drying gave less highly coloured extracts which allowed of a cleaner separation. Throughout, great difficulty was experienced in separating the hydrocarbons sufficiently for accurate determination.

The results show that small quantities of hydrocarbons were identified in the snuff itself and also in its constituents. The hydrocarbons in the plant materials are unlikely to form part of the structure of the plant and could possibly arise by being deposited on the outer surface—from native fires, etc. The ash was shown to contain a quantity of uncharred material, by loss of weight on low temperature ignition, (11 per cent in the aloe ash and 16 per cent in the Shi Tehava Misisi ash) which would undoubtedly contain hydrocarbons. These interesting findings are being considered further, and the results of the investigations will be published later.

It must be emphasised that native reluctance to divulge the traditional tribal secrets prevents us from forming a complete picture of the process used for preparing the snuff. The colour formed by mixing ash and tobacco as described is much paler than that of the snuff. The final product contains more hydrocarbons than can be accounted for by the amounts found in the ingredients, so possibly some further heat treatment takes place after mixing. Other materials are also

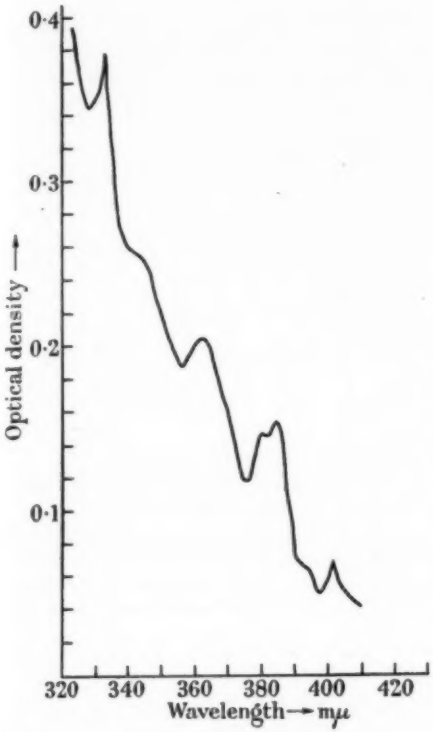


FIG. 5.—Spectrum of 3:4-benzpyrene in an extract of Zulu snuff.

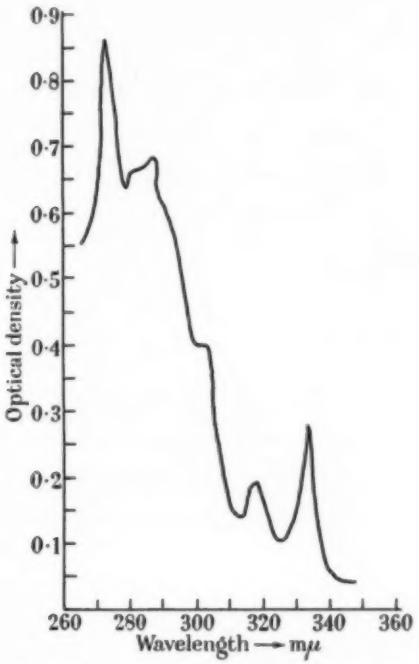


FIG. 6.—Spectrum of pyrene and fluoranthene in an extract of Zulu snuff.

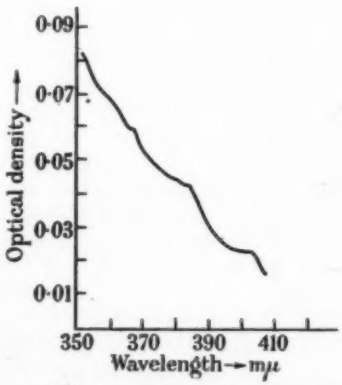


FIG. 7.—Spectrum of 3:4-benzpyrene in an extract of aloe stems.

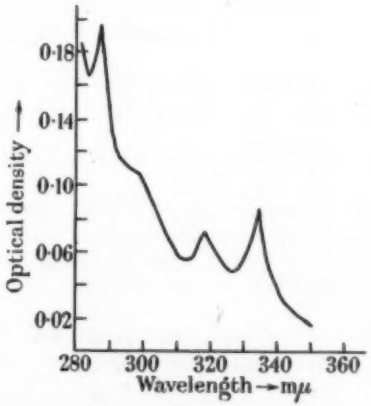


Fig. 8.—Spectrum of pyrene and fluoranthene in an extract of aloe stems.

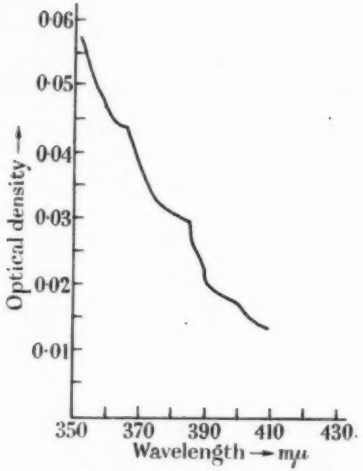


FIG. 9.—Spectrum of 3:4-benzpyrene in an extract of tobacco.

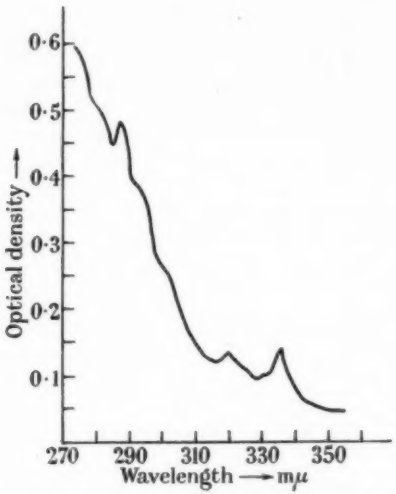


FIG. 10.—Spectrum of pyrene and fluoranthene in an extract of tobacco.

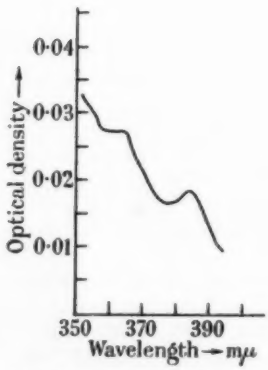


FIG. 11.—Spectrum of 3:4-benzpyrene in an extract of ash.

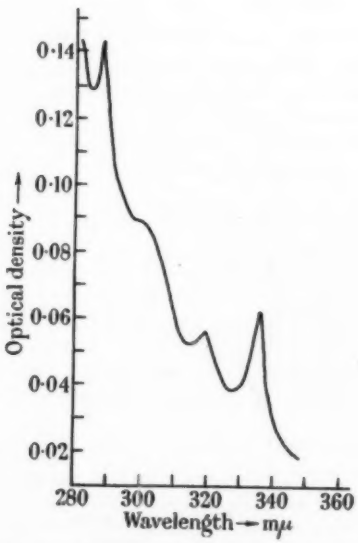


FIG. 12.—Spectrum of pyrene and fluoranthene in an extract of ash.

added, which we have not examined. The hydrocarbons were identified in the snuff, and it has been our experience to find these amongst the products of partial combustion of vegetable material, namely, tobacco and coal which is derived from plants by extremes of heat and pressure (Cooper and Lindsey, 1955; Cooper, 1954).

The range of proportions of the compounds compared with pyrene as unity is shown in Table IV.

TABLE IV.—*Relative Proportions of Hydrocarbons taking Pyrene as Unity*

| Hydrocarbons. | Zulu snuff. (1) | Zulu snuff. (2) | Venda snuff. (1) | Venda snuff. (2) | Cigarette smoke condensate | Chimney soot. |
|---|---|---|---|---|---|---|
| Anthracene | 0·12 | 0·09 | — | 0·11 | 1·0 | 0·2 |
| Fluoranthene | — | 1·4 | 1·5 | 1·6 | — | 1·3 |
| 3 : 4 Benzpyrene | 0·43 | 0·48 | — | — | 0·1 | 0·55 |
| 1 : 12 Benzperylene | — | 0·25 | — | — | — | 0·35 |

The relative proportions of the hydrocarbons in snuff are more like those in chimney soot rather than in tobacco smoke.

Of special interest is the fact that one of the compounds identified, 3 : 4-benzpyrene, is strongly carcinogenic and another, 1 : 12-benzperylene, is very weakly so. These results thus appear to strengthen the evidence in favour of a connection between the incidence of carcinoma of the nasal region amongst Bantu tribesmen and their habit of snuff-taking.

## SUMMARY

(1) The incidence of cancer of the respiratory tract in the Transvaal Bantu has been analysed from clinical material selected for radiotherapy during the 6-year period 1949–1954.

(2) In the rural African there is a remarkably high incidence of carcinoma of the nose and accessory sinuses, while lung cancer is relatively infrequent. As far as is known, the exceptionally high incidence of this tumour is found only in the South African Bantu.

(3) Most of these tumours were well differentiated squamous carcinomata and while the primary disease was always very advanced, lymph-node and distant metastases were infrequent.

(4) Tumours of the nasopharynx and larynx showed no significant racial predisposition.

(5) Among aetiological factors considered, personal habits seemed to be significant. Heavy cigarette smoking in the Bantu population is uncommon, due to economic considerations, whereas snuff-taking is extensively practised and this might well account for the high incidence of carcinoma of the nose and nasal sinuses.

(6) Samples of snuffs have been analysed and one of the compounds identified, 3 : 4-benzpyrene, is strongly carcinogenic, and another, 1 : 12-benzperylene, is very weakly so.

(7) These results are evidence in favour of a connection between carcinoma of the nose and paranasal sinuses in the Bantu people and their habit of snuff-taking.

The authors wish to acknowledge, the helpful advice given by Professor Sir Ernest Kennaway during the investigation.

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## REFERENCES

BERMAN, C.—(1951) ' Primary Carcinoma of the Liver.' London (H. K. Lewis).
BLACKLOCK, J. W. S., KENNAWAY, E. L., LEWIS, G. M. AND URQUHART, M. E.—(1954) *Brit. J. Cancer*, **8**, 40.
CAMPBELL, J. M. AND COOPER, R. L.—(1955) *Chem. & Ind. (Rev.)*, 64.
CLEMMESEN, J.—(1951) *Acta Un. int. Cancr.*, **7**, 24.
COOPER, R. L.—(1954) *Analyst*, **79**, 573.
*Idem* AND LINDSEY, A. J.—(1955) *Brit. J. Cancer*, **9**, 304.
DOLL, R.—(1951) *Acta Un. int. Cancr.*, **7**, 39.
EBERHARDT, J. L.—(1949) ' Survey of Housing and Family Conditions in Orlando Township.' Thesis, Univ. Witwatersrand.
HIGGINSON, J.—(1951) *Cancer*, **4**, 1224.
KHANOLKAR, V. R.—(1951) *Acta Un. int. Cancr.*, **7**, 51.
KOUWENAAR, W.—(1951) *Ibid.*, **7**, 61.
ÖHNGREN, L. G.—(1933) *Acta Otolaryng., Stockh.*, Supp. 19, 1.
SHAPIRO, M. P., KEEN, P., COHEN, L. AND DE MOOR, N. G.—(1955) *S. Afr. med. J.*, **29**, 95.
*Idem*, KEEN, P., COHEN, L. AND MURRAY, J. F.—(1953) *Brit. J. Cancer*, **7**, 45.
WATT, J. M. AND BREYER-BRANDWYK, M. G.—(1932) ' The Medicinal and Poisonous Plants of Southern Africa.' Edinburgh (E. & S. Livingstone).
WEDGWOOD, P. AND COOPER, R. L.—(1953) *Analyst*, **78**, 170.
WYNDER, E. L., GRAHAM, E. A. AND CRONIGER, A. B.—(1953) *Cancer Res.*, **13**, 855.

# THE HISTOLOGICAL STRUCTURE OF SOME HUMAN LUNG CANCERS AND THE POSSIBLE IMPLICATIONS FOR RADIOTHERAPY

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THE cells of stratified squamous epithelium, whether normal or neoplastic, generally remain in contact with one another. The vascular stroma on which their nutrition depends lies in contact with the epithelium but the capillaries do not penetrate between the individual cells. Tumours composed of these cells often grow in solid rods which, when seen in histological sections cut in a plane at right angles to their axes, appear as circular areas surrounded by stroma. In tumours of this kind the centres of the larger areas are necrotic and are surrounded by intact tumour cells which appear as rings.

It appeared that this particular structure might have an important bearing on the radiotherapy of such tumours, since there must exist a falling gradient in oxygen tension between the periphery and the centre of each tumour cord, and it is well known that cells which are anoxic at the time of irradition are generally much less damaged by a given dose of X- or $\gamma$-radiation than those which are well oxygenated (Gray *et al.*, 1953).

The magnitude of the oxygen gradient depends on the consumption of oxygen throughout the cell mass, which is not accurately known, but simple calculations show that the degree of anoxia of the cells which are more centrally situated might be very significant. For example, complete anoxia would be expected at the centre of a cord only 150$\mu$ in radius if the respiratory quotient throughout the tumour mass were 5·3 $\mu$l. of oxygen/mg. dry weight/hour.—the mean $Q_{O_2}$ of tissue slices from thirteen human tumours examined by Warburg (1930). We therefore undertook jointly a quantitative examination of many histological sections. We selected these from neoplasms which appeared to be growing in the manner described above. These tumours were mainly poorly differentiated squamous carcinomata.

Tumours of this type arise in any site where stratified squamous epithelium develops, and some 40 per cent of the bronchial carcinomata so far examined have this structure. It is mainly on these carcinomata that our calculations have been based, though a similar pattern of necrosis with approximately the same dimensions has been observed in squamous carcinomata arising in other sites, and in some carcinomata from other epithelia, *e.g.*, the stomach, the breast and the Fallopian tube.

## *Histological material*

Fresh operation specimens of carcinoma of the bronchus were fixed in 5 per cent formalin. 4–7$\mu$ sections were cut and stained with Erhlich's haematoxalin.

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*Qualitative examination of typical photomicrographs*

*Fig.* 1.—Transverse sections of tumour cords are seen, surrounded by stroma. No capillaries are visible among the tumour cells. All cords greater than about 180$\mu$ in radius have necrotic centres. This section is typical of areas of the tumour in which necrosis is not far advanced.

*Fig.* 2.—Large areas of necrosis are seen separated from the stroma by a band of tumour cells about 100$\mu$ wide.

*Fig.* 3.—In this section there are large areas of necrosis, but near the middle of the figure there is one instance in which the situation appears to be reversed, *i.e.*, the tumour cord appears to be growing independently of the stroma through a sea of necrosis. Examination under high power (Fig. 4) shows that this tumour cord is, in fact, nourished by a capillary running along its axis. This finding gives us confidence in our belief that capillaries have not been overlooked.

*Quantitative examination of typical photomicrographs*

Figures 6–10 present the results of a quantitative analysis of tumour pattern. Areas of an enlarged image of each section were measured by means of a planimeter and lengths by a cartographer's wheel. In the case of approximately isodiametric tumour cords, the radii $r$ and $R$ (*cf.* Fig. 5) of the cylinders having the same cross-sectional area as the necrotic central region, and the total area of the cord respectively, were computed. When a sea of necrosis was separated from the stroma by a band of tumour cells as in Fig. 2 and 3, the mean width of the band was estimated as the ratio of the area to the length measured along the middle of the band. In each of Fig. 6–10 values of $R$ are plotted as abscissae. The open circles show the radius of the necrotic centres. The full circles show the maximum distance of a tumour cell from stroma, this being measured either as the total radius of a cord which has no necrotic centre, or as $(R - r)$. In the case of cords which have no necrotic centre the full circles necessarily fall on the 45° line. Each cord having a necrotic centre is defined by a pair of points, a full and an open circle. Those areas of necrosis which exceed 2 mm. in any dimension are classified to the right as " very large ", and the full circles denote the thickness of the band of tumour cells which borders them. Fig. 9 and 10 refer to different areas of the same tumour. The remaining figures refer to different tumours. In some cases a total depth of 500$\mu$ of tumour tissue was sectioned at 25$\mu$ intervals. The histological patterns seen in the individual sections have not yet been measured, but there is no obvious change in the dimensions of the pattern throughout this depth of tumour material.

*Summary of results*

Some 160 tumour areas have been measured. With only one exception it has been found :

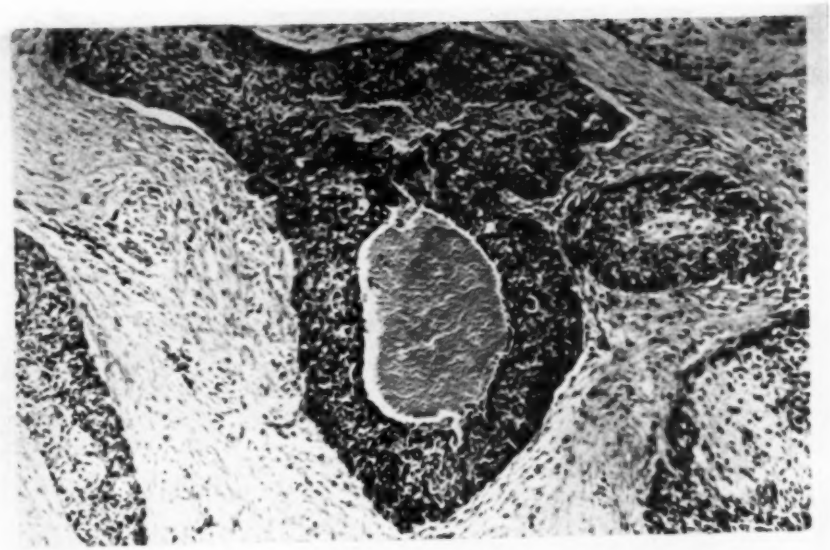
(1) That there is no tumour cord more than 200$\mu$ in radius which is without central necrosis ;

(2) no central necrosis is seen in any tumour cord less than 160$\mu$ in radius ;

EXPLANATION OF PLATES

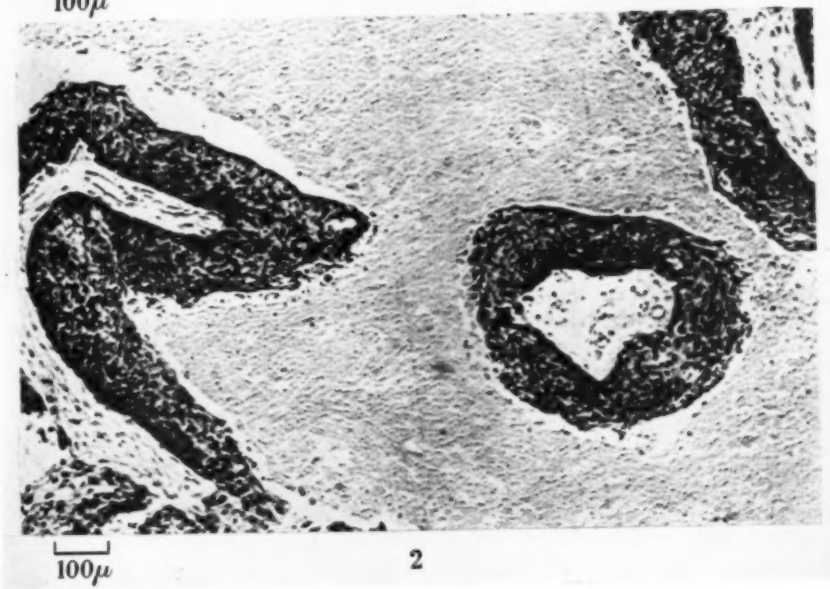
FIG. 1–3.—Squamous carcinoma of the bronchus. Fixed 5 per cent formalin and stained with haematoxalin and eosin.

FIG. 4.—Enlarged view of tumour cord seen at the centre of Fig. 3.



100$\mu$

1



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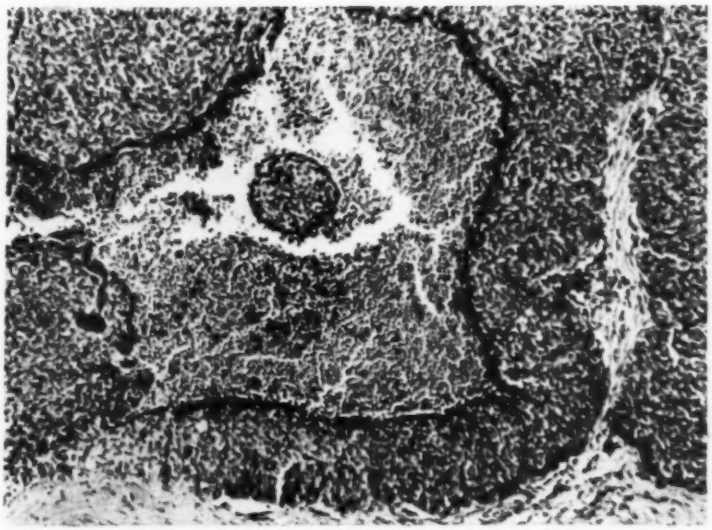
Thomlinson and Gray.



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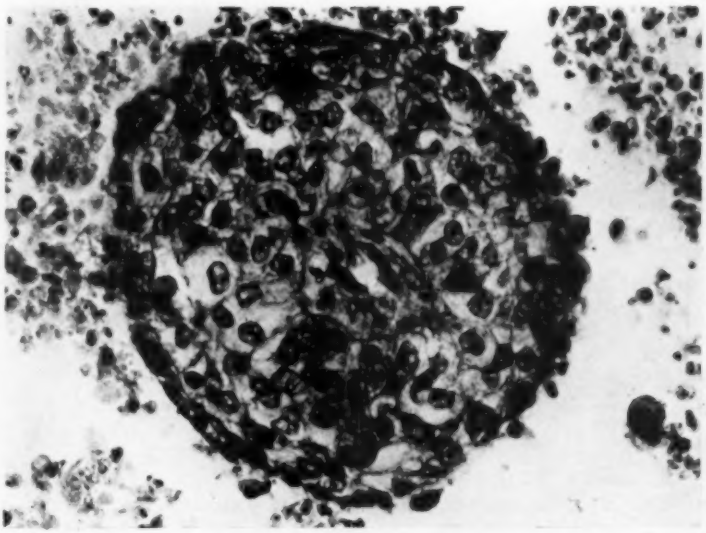

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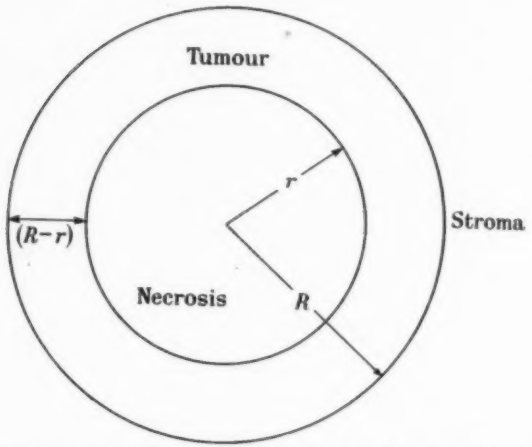


FIG. 5.—Diagrammatic representation of a tumour cord for comparison with Fig. 6–10.

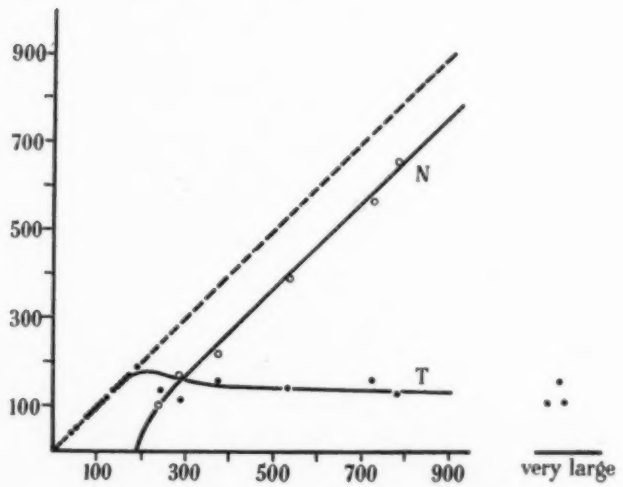


FIG. 6.

FIG. 6–10.—Dimensions of tumour cord $R$, central necrosis $r$, and region of tumour which has not yet become necrotic ($R - r$). The dimensions (in microns) are those seen in sections of carcinoma of the bronchus fixed with 5 per cent formalin and stained with haematoxalin and eosin. *Abs.*: Radius $R$ of interface between tumour cord and stroma. *Ords.*: Radius of central necrosis $r$ and thickness of cylindrical shell of tumour ($R - r$). ● Tumour (curve T). ○ Central necrosis (curve $N$). Fig. 6 case 5162/53; Fig. 7 case 4127/54; Fig. 8 case 4084/53; Fig. 9 case 889/53; Fig. 10 case 889/53, different region of the same tumour as Fig. 9.

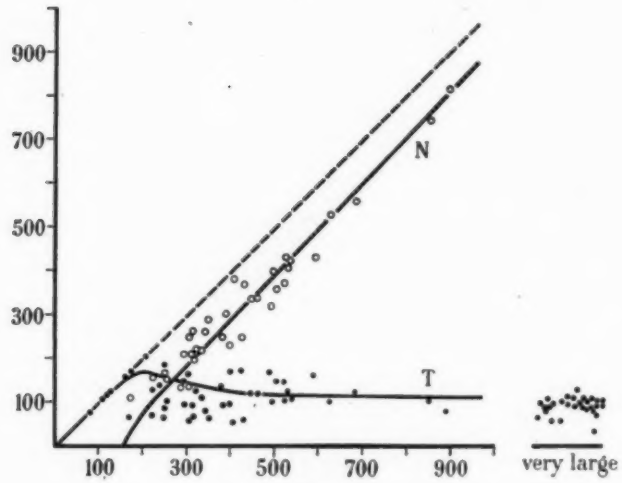
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FIG. 7.

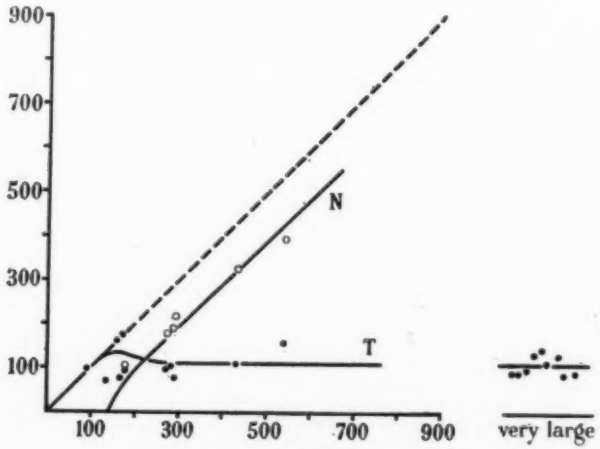
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FIG. 8.

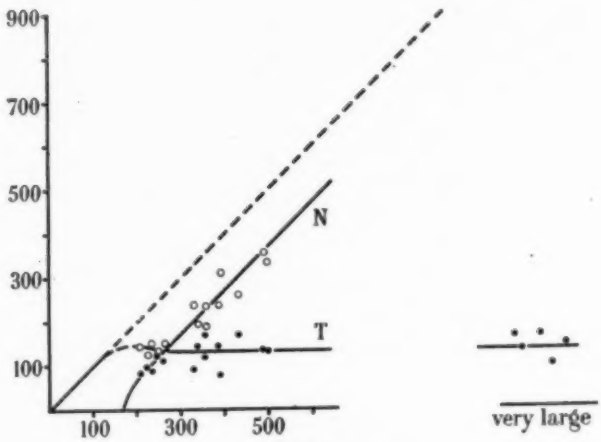
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FIG. 9.

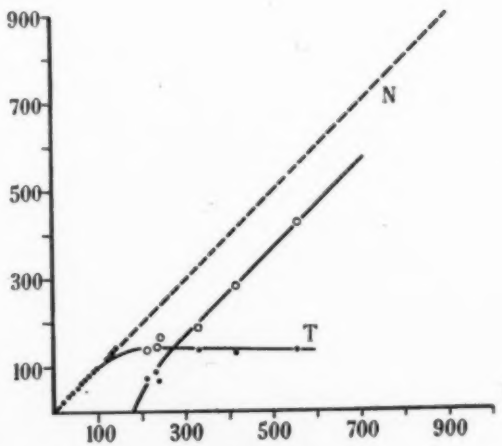
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FIG. 10.

(3) however great the radius of the necrotic centre, the thickness of the surrounding sheath of tumour cells never exceeds 180$\mu$—*i.e.*, no apparently intact tumour cell is seen more than 180$\mu$ from the stroma.

An apparent exception consisted of a cord having a radius of 500$\mu$ which showed no evidence at all of central necrosis, but it was found that the section had been cut almost through the distal end of the cord. At 100$\mu$ deeper in the tissue there was abundant stroma through which the tumour cells could derive their nutrients, and at 100$\mu$ proximally the cord showed a necrotic centre. This, therefore, does not constitute an exception in principle.

Factors which probably contribute to the scatter of the observed values of $(R - r)$ about the mean are :

(1) In many cases the tumour cord is not running exactly perpendicular to the plane of the section. In such cases the observed values of $(R - r)$ will be greater than the true values.

(2) Factors, such as compression of blood vessels, which decrease the blood supply to the whole area and therefore decrease the nutrient concentration at the periphery of the individual cords. In such cases the belt of non-necrotic tumour cells $(R - r)$ will be thinner than when the periphery is adjacent to stroma which is well supplied with blood.

*Computed nutritional gradients*

For diffusion into a cylindrical mass of metabolising tissue, it may be shown* that :

$$C = C_0 - \frac{M}{4D}\left[(R^2 - r^2) - a^2 \log_e\left(\frac{R^2}{r^2}\right)\right] \quad . \quad \text{Equation (1)}$$

where $a$ is defined by the equation

$$a^2\left[1 + \log_e\left(\frac{R^2}{a^2}\right)\right] = R^2 - R_{crit}{}^2 \quad . \quad . \quad \text{Equation (2)}$$

and

$$R_{crit} = \sqrt{\frac{4DC}{M}} \quad . \quad . \quad . \quad \text{Equation (3)}$$

In these equations,

$C$ = concentration of metabolite at radius $r$.
$C_0$ = concentration of metabolite at the surface of the cylinder, radius $R$.
$M$ = metabolite consumed per unit volume per second (assumed to be independent of $C$).
$D$ = coefficient of diffusion.
$R_{crit}$ = the critical value of the radius $R$ which is such that the concentration of the metabolite just reaches zero at the centre.
$a$ = radius at which the concentration of metabolite reaches zero when the outer radius of the cylinder $R > R_{crit}$.

* *cf.*, for example, A. V. Hill, who treats the dynamic as well as the equilibrium cases of diffusion of oxygen into plane and cylindrical elements of tissue, and the outward diffusion of lactic acid.

When $R < R_{crit}$ equation (1) reduces to

$$C = C_0 - \frac{M}{4D}(R^2 - r^2) \quad . \quad . \quad . \quad \text{Equation (4)}$$

In these formulae it is assumed that distances are measured in centimetres and the diffusion constant in $cm^2$/sec. The quantities $M$ and $C$ always occur as the ratios $M/C$ or $M/C_0$ which express the fraction of the amount of metabolite contained in unit volume of medium which is consumed per second. Any convenient unit may be chosen as a measure of the amount of metbolite, provided the same unit is used for $C$ and $M$.

Since we are here concerned with the diffusion and consumption of oxygen it is convenient to express the rate of consumption of oxygen in terms of the respiratory rate $Q_{O_2}$ measured as microlitres of oxygen consumed per milligram dry weight per hour.

It is also convenient to express the concentration $C$ in terms of the partial pressure of oxygen in mm. Hg. in a gas phase with which the liquid or tissue is in equilibrium.

Thus, if the unit quantity of oxygen is 1 ml. of gas at N.T.P.,

$$C = \frac{p}{760} \times s$$

where $s$ is the solubility of oxygen in tissue at the given temperature, expressed as ml. oxygen per ml. of tissue.

$$M = \frac{f \times Q_{O_2}}{3600} \text{ ml. oxygen per ml. tissue per second}$$

where $f$ = dry weight/wet weight of tissue which is assumed to have unit density in the wet state.

$D$ = coefficient of diffusion at the given temperature in $cm.^2sec.^{-1}$.

Thus,

$$\frac{DC}{M} = D \times \frac{p}{760} \times s \times \frac{3600}{f \times Q_{O_2}} = 4{\cdot}73 \frac{D \times s \times p}{f \times Q_{O_2}}$$

None of the four constants $D$, $s$, $f$ and $Q_{O_2}$ has been measured for the tissue composing the lung tumours which we have examined. For the purpose of making an approximate estimate of the oxygen gradients to be expected within the tumour cords we have used the following values :

$f$ = 0·25, implying that the cells contain 75 per cent water, by weight.

$s$ = 0·024 × 0·75 = 0·018 being the amount of oxygen dissolved at 37° C. in the water content of 1 ml. of tissue.

$Q_{O_2}$ = 5·2 $\mu$l. $O_2$/mg. dry weight/hour, being the mean of 13 human tumours measured by Warburg (1930).

$D_{37°C.} = 2{\cdot}0.10^{-5}$ $cm.^2sec.^{-1}$. This figure is intermediate between the values for water and muscle (Krogh, 1924).

Then

$$\frac{DC}{M} = \frac{4{\cdot}73 \times 2{\cdot}10^{-5} \times 0{\cdot}018}{0{\cdot}25 \times 5{\cdot}2} \times p = 1{\cdot}31.10^{-6}.p.$$

so that when the partial pressure of oxygen at the surface of the tumour cord is 40 mm. Hg.

$$R_{crit} = \sqrt{\frac{4DC}{M}} = \sqrt{4 \times 1{\cdot}31.10^{-6} \times 40}\ \text{cm.} = 145\ \text{microns.}$$

Fig. 11 shows the distribution of partial pressure of oxygen through cylinders of radius 100$\mu$, 145$\mu$ (critical radius) and 300$\mu$. In Fig. 12, $r$ and $(R - r)$ are plotted against $R$ for direct comparison with Fig. 6–10.

Qualitatively, the distribution of concentration of a katabolite, such as lactic acid, would be as shown by the broken line in Fig. 11. If the onset of necrosis is set by deficiency of a nutrient (and not by accummulation of a katabolite) then the

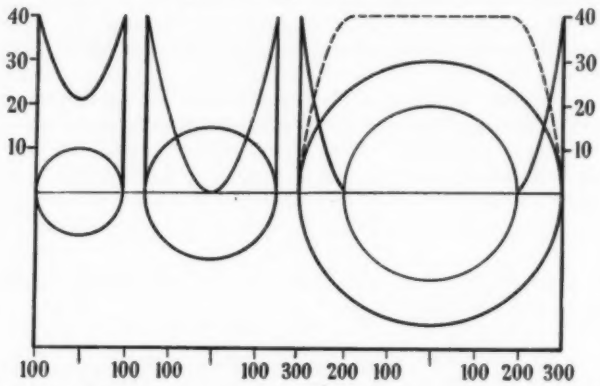


FIG. 11.—Distribution of partial pressure of oxygen through a cylinder of respiring tissue assuming $Q_{O_2} = 5{\cdot}2$ µl. $O_2$/mg. dry weight/hour and $D_{37°C.} = 2{\cdot}0.10^{-5}$ cm.$^2$sec.$^{-1}$. *Abs.:* Distance from axis of cylinder in microns. *Ords.:* Partial pressure of oxygen in mm. Hg. Broken line gives diagrammatic representation of the distribution of a catabolite.

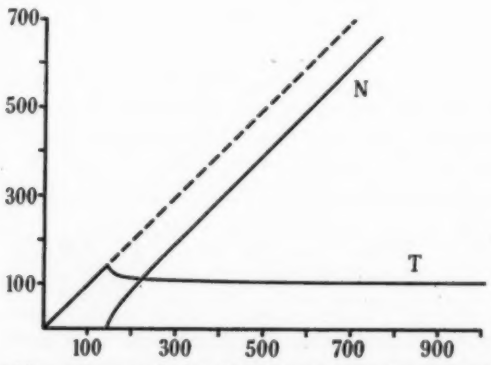


FIG. 12.—Theoretical form of curve relating the radius of a central anoxic cylinder to the outer radius of a cylinder of metabolising tissue. Calculations assume $Q_{O_2} = 5{\cdot}2$ µl. $O_2$/mg. dry weight /hour. and $D_{37°C.} = 2{\cdot}0.10^{-5}$ cm.$^2$sec.$^{-1}$. All radii are measured in microns. *Abs.:* Outer radius of cylinder. *Ords.:* Curve $N$—radius of central cylinder of necrosis. Curve $T$—thickness of zone which is supplied with oxygen. For comparison with shapes of curves in Fig. 6–10.

concentration of the katabolite will be uniform from $R = 0$ to $R = a$, and then fall to zero at $r = R$, unless the katabolite happens also to be a product of cellular disintegration, in which case its concentration may reach a maximum at smaller values of $r$ than $a$. At present we lack the data required for a quantitative evaluation of the katabolite concentration distribution.

*Comments*

1. The histological structure of those tumours included in this analysis conforms closely in pattern to that which would be expected as a result of the peripheral proliferation of tumour cords which are devoid of capillaries and which comprise cells nourished by diffusion of metabolites inwards from the immediately surrounding stroma. Since no arbitrary constants have been introduced into the calculation of oxygen gradients the results of these calculations show that the scale of the observed histological pattern is of the order to be expected if the supply of oxygen were the limiting factor which determines the onset of necrosis, but this numerical agreement is not advanced as evidence that the cells at the centre in fact die, through lack of oxygen.

Referring to Fig. 6 and 10, it will be seen that the observed critical radii are approximately 190, 170, 140, 170 and 175$\mu$ respectively, averaging 169$\mu$. This figure is to be compared with the calculated value of 145$\mu$.

No great significance can be attached to this rather close numerical agreement on account of the uncertainties in our knowledge of the various constants used in the calculations. The diffusion constant could differ by 30 per cent in either direction from the value we have assumed. Of the remaining constants the respiratory quotient is probably the least certain for the following reasons :

(*a*) The respiratory quotient of the cells composing those tumour cords which have been examined microscopically is unknown. We have not been able to find in the literature any measurements relating to human lung cancer. The value 1·3 $\mu$l. of oxygen/mg. wet weight/hour which we have used in our calculations is derived from the mean value of the $Q_{O_2}$ of tissue slices from thirteen human tumours examined by Warburg (1930) which ranged from 2–8 $\mu$l. of oxygen/mg. dry weight/hour. We have assumed a wet to dry weight ration of 4 : 1. Warburg's series did not include carcinoma of the bronchus. Moreover, the proportion of necrotic tissue and of non-cancerous tissue in the samples is not known. Dickens and Patey (1930), who examined the metabolism of tumour tissue and normal tissue from seventeen patients suffering from mammary carcinoma, found wide variations in the $Q_{O_2}$, $Q^{O_2}_{CO_2}$ and $Q^{N_2}_{CO_2}$ of different tumours. These authors established a cellularity index which gave a rough measure of the proportion of tumour cells in the tissue slices used for metabolic measurements. When their results are charted there is an evident correlation between metabolic activity and cellularity index for each of the three quotients, and in the case of respiration it is apparent that the best estimate for the average $Q_{O_2}$ of the tumour cells would be about double the average value for the whole tissue slice, *viz.*, $\sim$10 $\mu$l. of oxygen/mg. dry weight/hour.

(*b*) Our calculations assume that the respiratory quotient is independent of oxygen tension. This is known to be true down to low oxygen tensions for muscle, but no observations have been found relating to tumour tissue.

If quantitative data were obtained for the respiration and for the aerobic and anaerobic glycolysis of a particular human tumour, as a function of the concentra-

tion of oxygen and of sugar in the nutrient medium, the metabolic gradients for oxygen, sugar and lactic acid could be approximately calculated; comparison with the histological appearance of the tumour might then show the probable cause of the onset of necrosis which remains at present a matter of conjecture.

*Implications for radiotherapy*

Whatever determines the onset of necrosis, the concentration of oxygen must, in the absence of capillaries among the tumour cells, be lower towards the centre than at the periphery of the tumour cord. It is probable that the gradients depicted in Fig. 11 are correct as to order of magnitude. On account of their lower oxygen tension at the time of irradiation the innermost viable tumour cells are likely to be much more radioresistant than those at the periphery of the cord. The radiosensitivity may also be influenced—possibly to a much greater extent—by other aspects of the peculiar metabolic conditions of the innermost cells. In the absence of irradiation these cells would probably die since, as a result of the proliferation of the outer cells, they will become further removed from stroma than the critical distance compatible with survival. A dose of radiation, which suffices to kill the outer cells but not the more resistant inner cells, will, however, increase the supply of nutrients to the inner cells. If they have retained their reproductive integrity during the period of under-nourishment, they may once again proliferate. In this connection, we have been interested to note that Caspersson and Santesson (1942) observed by UV microscopy that the cells comprising tumour cords showed a gradation in cytological and cytochemical structure from the periphery to the centre of the cord and interpreted this gradient in terms of the supply of nutrients by diffusion. They observed that the cells composing an encapsulated tumour showed no evidence of proliferative activity, but became transformed into typical proliferating cells on breaking through the capsule. This suggests that cells may remain dormant, through lack of nutriment, for a considerable time without losing their ability to proliferate.

The relation between oxygen tension and radiosensitivity of many types of cell, both normal and malignant, is such that a sufficiently large increase in oxygen tension at the periphery of the tumour cord would be likely to secure a greater differential damage to tumour as compared with that to tissues in the body which are normally well oxygenated. This has been discussed elsewhere (Gray *et al.*, 1953) in connection with animal experiments which have shown that the administration of oxygen to an animal at the time of irradiation can increase the effectiveness of a given dose by a factor of 1·5 to 2 as judged by the subsequent regression of the tumour, while only slightly increasing the skin reaction.

It is important, however, to bear in mind that in principle the differential gain in sensitivity is linked with a particular histological structure and not specifically with malignancy. The case of bone is of special interest in this connection. Scott remarked (Gray *et al.*, 1953) upon an apparent increase in the incidence of bone necrosis in the legs of mice which had carried inoculated tumours, when they were irradiated while the animals were breathing oxygen. This matter has been carefully investigated by Howard-Flanders and Wright (1955), who find that oxygen respiration leads to an increased radiation damage to the growing vertebrae of the tails of very young mice. In these bones the dividing cells, whose injury was probably mainly responsible for the reduced growth rate of the tail after irradiation, were situated approximately in the mid-plane of epiphyseal cartilage 200$\mu$ thick,

and were therefore separated by about 100$\mu$ from the nearest capillaries (for providing this information we are greatly indebted to Dr. Wright.) The structure of these bones is thus essentially similar to, and on approximately the same scale as, the tumours which we have described. Unfortunately the $Q_{O_2}$ of the epiphyseal cartilage of young mouse tails is not known. These bones may nevertheless provide very convenient models for the study of possible methods of controlling oxygen tension in relation to the radiotherapy of tumours which have the histological pattern of the lung carcinomas discussed in this paper.

## REFERENCES

CASPERSSON, T. AND SANTESSON, L.—(1942) *Acta Radiol., Suppl.*, 46.

DICKENS, F. AND PATEY, D. H.—(1930) *Lancet*, ii, 1229.

GRAY, L. H., CONGER, A. D., EBERT, M., HORNSEY, S., AND SCOTT, O. C. A.—(1953) *Brit. J. Radiol.*, **26**, 638.

HOWARD-FLANDERS, P. AND WRIGHT, E. A.—(1955) *Nature*, **175**, 428.

KROGH, A.—(1924) "The anatomy and Physiology of Capillaries." New Haven, Conn. (Yale University Press), p. 196.

WARBURG, O. (1930). "The Metabolism of Tumours." English Edition (Dickens), London (Constable & Co. Ltd.), Preface, p. 6.

# GASTRIC POLYPS COMPOSED OF INTESTINAL EPITHELIUM

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METAPLASIA of the gastric mucous membrane to an intestinal type of epithelium may be very extensive (Magnus, 1937 ; Stout, 1945 ; Morson, 1955*a*). Uncommonly, almost the whole lining of the stomach may be affected. It would not be surprising, therefore, if polyps composed of an intestinal type of epithelium were found in the stomach.

A series of 12 gastric polyps have been investigated. Five of these were obtained from the records of the Bland-Sutton Institute of Pathology at the Middlesex Hospital and seven from a series of 160 gastrectomy specimens removed for carcinoma (an incidence of 4·4 per cent) which have been collected by the writer over the past four years. It is of interest that a control series of 80 gastrectomy specimens removed for benign gastric and duodenal ulcer did not contain a single polyp.

Out of the total of 12 polyps in this series, 7 are composed of true gastric mucosa, and 5 of an intestinal type of epithelium. Three of the latter show histological evidence of malignant transformation. That some gastric polyps are composed of intestinal epithelium, and may become malignant, is evidence in support of the conclusion made elsewhere (Morson, 1955*b*) that some gastric carcinomas arise from areas of intestinal metaplasia in the gastric mucosa.

The identification of the intestinal type of gastric polyp is based on the following characteristics : (1) The presence of goblet cells. These are columnar cells containing a droplet of mucus which stains blue with Ehrlich's acid haematoxylin and red with Southgates' modification of Mayers' mucicarmine method. Goblet cells are not found in normal gastric mucosa ; they may only be seen in association with areas of intestinal metaplasia (Magnus, 1937 ; Morson, 1955*a*). In the intestinal type of gastric polyp goblet cells are easily found. However, as epithelial hyperplasia in a polyp of this type becomes more severe the goblet cells become fewer. (2) The lining epithelium of the tubules composing the intestinal type of gastric polyp bears a " striated " or " brush " border. This feature is not seen in normal gastric epithelium, but is characteristic of epithelial cells of intestinal type. It may be seen with difficulty in ordinary haematoxylin and eosin preparations, but can be more clearly demonstrated by stains such as the periodic acid routine described by McManus (1948). (3) Paneth cells may be found in small numbers in the intestinal type of gastric polyp. They contain coarse granules which are stained bright red by eosin. Phosphotungstic acid haematoxylin stains the granules of Paneth cells a deep purple colour, and apart from being a specific reaction, has the advantage that mucicarmine can be used as a counterstain to demonstrate goblet cells (Magnus, 1937). (4) Argentaffin cells are a characteristic of intestinal epithelium. They are to be found in gastric polyps composed of intestinal epithelium in small numbers, and may be stained either by Masson's silver impregnation method or by the diazo method described by

Jacobsen (1939). (5) The features of true gastric mucosa are almost completely absent from the intestinal type of gastric polyp. Furthermore, the mucous membrane surrounding this type of polyp shows complete metaplasia to an intestinal type of epithelium in all the cases in this series. Occasionally tubules reminiscent of pyloric glands or the superficial type of gastric epithelium may be seen, but these are difficult to find and in any case do not appear to be taking part in the neoplastic process.

Seven out of the twelve polyps in this series are composed of true gastric epithelium. One of these was removed from a stomach bearing multiple small sessile adenomata, all of similar histology. They showed overgrowth of the superficial layer of the gastric mucosa and a core containing gastric glands mixed up with large dilated tubules lined by columnar epithelium. In the other six polyps composed of true gastric epithelium the cell type involved in all cases is that found in the superficial layer of the gastric mucosa. This consists of tall columnar cells with basal nuclei and a clear cytoplasm. When epithelial hyperplasia in this type of polyp becomes severe the columnar epithelium tends to become more cubical and the cytoplasm stains more darkly. However, no goblet, Paneth or argentaffin cells can be seen. Also, there is no evidence of intestinal metaplasia in the polyp or the adjacent mucous membrane. Only one of the polyps of this type appeared to be undergoing malignant change.

Three examples of the intestinal type of gastric polyp are described and illustrated with microphotographs. In each case a complete description is given of the macroscopic and microscopic appearances of the tumour, together with some comments on the state of the adjacent mucous membrane. A study of the latter gives further evidence of the type of epithelium from which the polyp has arisen.

*Case No.* 1. W. B. Age 63. Male.

*Description of specimen.*—Total gastrectomy with attached spleen and greater omentum. There is a protuberant carcinoma, $2\frac{1}{2}$ inches in diameter, involving the entire circumference of the gastric cardia. In addition there is a polyp, $\frac{1}{2} \times \frac{1}{4}$ inch, lying close to the pyloroduodenal junction. This appears to be confined to the mucosa.

*Histology* (Fig. 1, 2, 3).—Sections of the growth at the cardia show a moderately well-differentiated adenocarcinoma. The polyp near the pyloroduodenal junction is benign. It is composed of tubules lined by hyperplastic columnar epithelium (Fig. 1). Among these scattered goblet and Paneth cells may be seen. Argentaffin cells are also present.

A study of the gastric mucosa of this stomach as a whole was made by examining long strips of mucous membrane rolled up in the form of a swiss roll (Magnus, 1937 ; Morson, 1955*a*). This method enables large areas of the gastric mucosa to be examined in one section. In this case the mucosa of the entire stomach, with the exception of the fundus, shows very extensive metaplasia to an intestinal type of epithelium. There is only a slight extent of intestinal metaplasia at the fundus. Further, the mucosa immediately adjacent to the polyp shows complete intestinal metaplasia. There is also complete continuity between the polyp and its surrounding mucous membrane (Fig. 1).

The polyp itself is composed of tubules lined by hyperplastic columnar epithelium (Fig. 1). Scattered goblet and Paneth cells are present throughout the

tumour (Fig. 2, and 3). The epithelium lining the tubules contains large irregularly shaped nuclei, and an increased number of mitoses is apparent. It is notable that the histological characteristics of true gastric mucosa are almost completely absent. At the centre of the polyp there are one or two small tubules reminiscent of pyloric glands (Fig. 1), but apart from this the epithelium is entirely of intestinal type. The histological appearances of the polyp and the mucosa of this stomach suggest not only that the polyp is composed of intestinal epithelium, but that it has arisen from an area of intestinal metaplasia in the gastric mucosa.

*Case No.* 2. R. B. Age 64. Female.

*Description of specimen.*—Local excision of stomach wall from the region of the pylorus. The specimen is about 1½ inches in diameter and bears a pedunculated polyp about ¾ inch in diameter.

*Histology* (Fig. 4).—Sections show a pedunculated papilloma composed of tubules lined by hyperplastic columnar epithelium. There is irregularity in the shape and size of nuclei, and many mitoses can be seen. Further, there appears to be early invasion of the stalk. The appearances suggest that the polyp has become malignant.

As this tumour was excised locally only the mucosa in its immediate neighbourhood is available for study. This shows complete metaplasia to an intestinal type of epithelium (Fig. 5). No true gastric mucosa can be seen. Numerous goblet and Paneth cells are present and occasional argentaffin cells can be identified at the bases of the tubules. In addition the epithelium lining the tubules appears to be very hyperplastic and there is one area suggestive of malignant change *in situ*. The metaplastic mucosa shows continuity with the epithelial tubules composing the polyp.

A study of the histology of this polyp reveals that it is composed of tubules lined by very hyperplastic columnar epithelium. This epithelium shows only scattered goblet cells which tend to be very few where the epithelium is most hyperplastic. No Paneth or argentaffin cells can be seen in the polyp, but the epithelium does appear to have a striated border. Even this is not very clearly

EXPLANATION OF PLATES

FIG. 1.—Case 1. Gastric polyp composed of rather irregular tubules lined by hyperplastic columnar epithelium. The adjacent mucosa is atrophic and shows intestinal metaplasia. Note the darkly stained goblet cells. No true gastric mucosa can be seen. Haematoxylin and eosin. × 10.

FIG. 2.—Case 1. Tubules in the polyp lined by hyperplastic columnar epithelium and showing goblet cells. Phosphotungstic acid haematoxylin and mucicarmine. × 250.

FIG. 3.—Case 1. High power view of polyp to show a Paneth cell at the top and a goblet cell in the left lower corner. Phosphotungstic acid haematoxylin and mucicarmine. × 600.

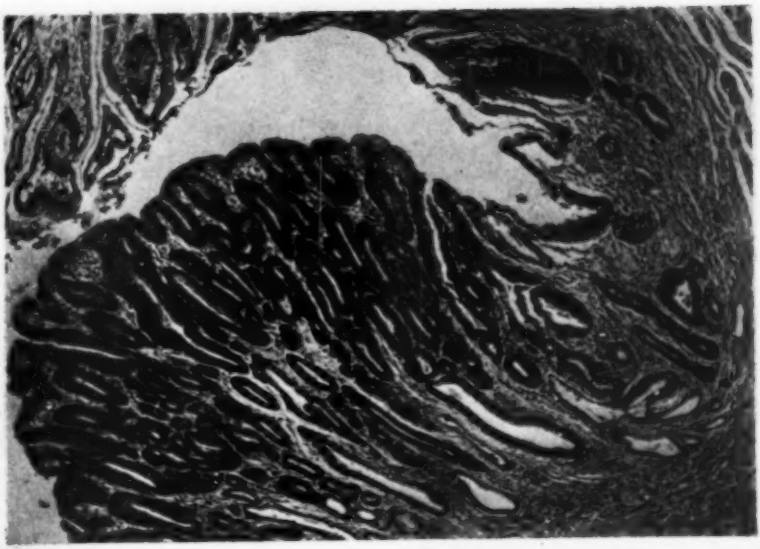
FIG. 4.—Case 2. Gastric polyp and its adjacent mucosa. The appearances are very similar to those of an intestinal type of polyp. Haematoxylin and eosin. × 8.

FIG. 5.—Case 2. Mucosa immediately adjacent to the polyp showing complete intestinal metaplasia. Haematoxylin and eosin. × 90.

FIG. 6.—Case 3. Edge of sessile polyp. The mucosa on the left shows complete intestinal metaplasia and is continuous with the adenomatous area on the right. Note the numerous goblet cells and absence of any of the characteristics of true gastric mucosa. Haematoxylin and eosin. × 40.

FIG. 7.—Case 3. High power view of tubules lined by hyperplastic epithelium and containing a few goblet cells. These are disappearing and are seen as small droplets lying close to the lumen of the tubule. Mucicarmine. × 250.

FIG. 8.—Case 3. High power view of tubule lined by hyperplastic epithelium showing numerous mitoses. Despite the hyperplasia goblet cells are still present. Mucicarmine. × 400.



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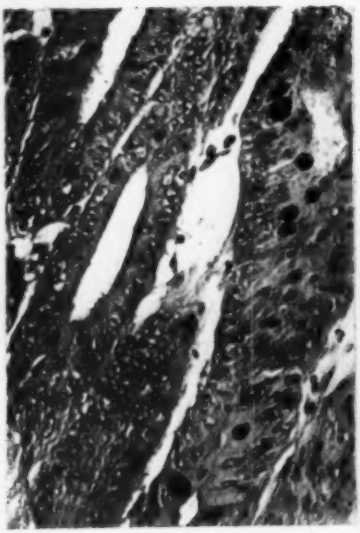

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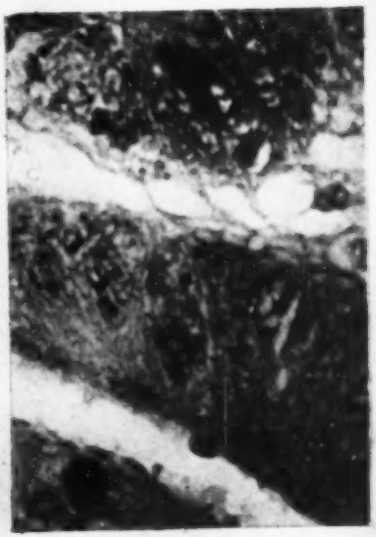

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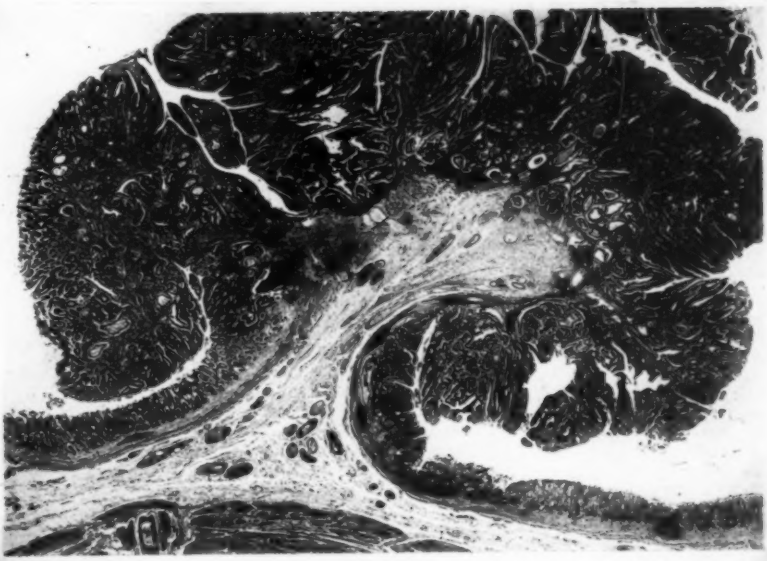

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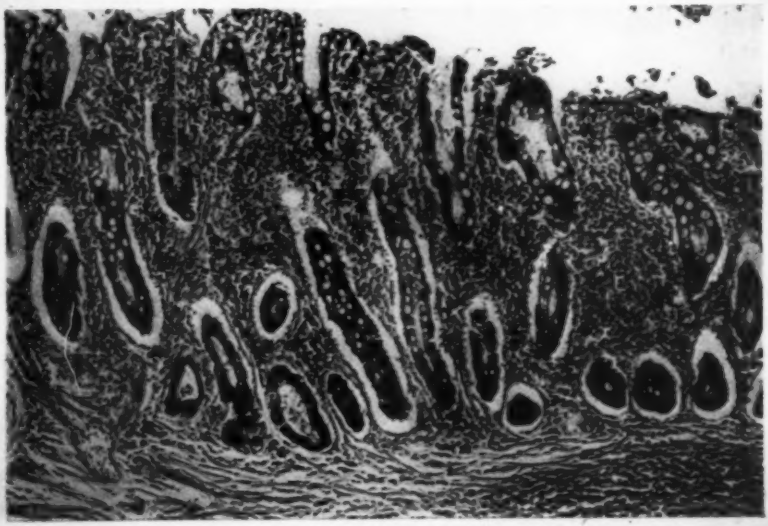

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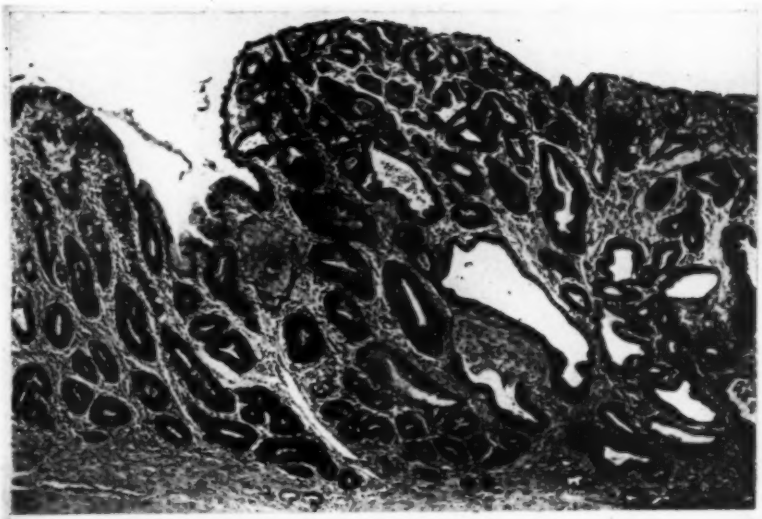

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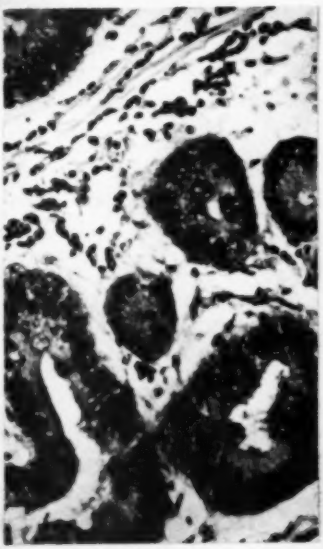

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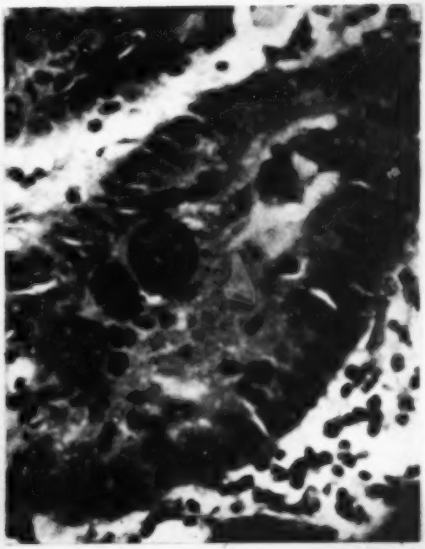

8

shown. Although this polyp shows indefinite characteristics of intestinal epithelium it does not have the features of true gastric epithelium. Moreover, it is surrounded by a mucosa showing complete intestinal metaplasia. The observation that only a few goblet cells can be demonstrated in the tumour and no Paneth or argentaffin cells is explained by the loss of differentiation which goes with malignant change and obscures the characteristics of the parent tissue. All the same, the histological evidence suggests that this papilloma is composed of intestinal epithelium and has arisen from an area of intestinal metaplasia in the gastric mucosa.

*Case No.* 3. A. R. Age not known. Female.

*Description of specimen.*—Partial gastrectomy. There is an ulcer, 1 inch in diameter, lying close to the pyloro-duodenal junction. In addition there is a sessile polyp, also 1 inch in diameter, on the lesser curvature of the stomach $2\frac{1}{2}$ inches from the pyloro-duodenal junction and $1\frac{1}{2}$ inches from the proximal limit of excision.

*Histology* (Fig. 6, 7, 8).—Sections show an adenocarcinoma at the pylorus. The sessile polyp on the lesser curvature shows adenomatous hyperplasia in epithelium of intestinal type. There are also changes suggestive of early malignant change.

The mucous membrane of this gastrectomy specimen was examined by the " swiss-roll " method referred to in Case No. 1. Long strips of mucosa taken from the entire length of the lesser curvature and posterior walls of the stomach show complete metaplasia of the mucosa to an intestinal type of epithelium. Moreover, the polyp on the lesser curve is completely surrounded by metaplastic mucosa (Fig. 6). There is continuity between the epithelium of the polyp and its adjacent mucous membrane. Neither show any of the features of true gastric epithelium.

The polyp is composed of tubules lined by reduplicated and hyperplastic epithelium. At a number of points there is irregularity in shape and size of nuclei with an increased number of mitoses. The appearances suggest early malignant change. Goblet cells are scattered throughout the tumour, but they tend to disappear at points where epithelial hyperplasia is most advanced. Occasional Paneth and argentaffin cells are present, but these can only be seen at the bases of the tubules and do not appear to be taking part in the neoplastic process. The appearances are those of an intestinal type of epithelium, and in view of the complete absence of any of the features of true gastric epithelium in the polyp or in its surrounding mucosa, it is probable that the adenomatous hyperplasia has occurred in an area of intestinal metaplasia.

## DISCUSSION

Brunn and Pearl (1943) recognize two types of gastric polyp—congenital and acquired. They state that the latter may be due to chronic irritation and mention that some gastric polyps contain features (goblet and Paneth cells) normally associated with intestinal epithelium. Spriggs (1943) found " grades of mucoid degeneration " in some of his epithelial polyps, but he does not enlarge on this point and none of his illustrations suggest that any of the tumours in his series were entirely composed of intestinal epithelium. Other authors who have studied gastric polyps include Stewart (1913, 1929), Gleave (1923), Miller, Eliason and Wright (1930) and Willis (1948). None of them mention that gastric polyps

may contain epithelium of intestinal type. However, Jarvi and Lauren (1951) show a microphotograph of a polyp apparently composed entirely of intestinal epithelium. They state that the whole of the epithelium of this polyp bears a striated border and shows scattered goblet cells. These features are characteristics only of intestinal epithelium. More recently Mulligan and Rember (1954) state that six out of 33 examples of their " intestinal type " of gastric carcinoma arose from a glandular polyp. It is justifiable to infer that these polyps were composed of intestinal epithelium. It is curious that the existence of an intestinal type of gastric polyp should have received so little consideration in the literature. The reasons for this are probably twofold. Firstly, gastric polyps are uncommon. Compared with the frequency of polyps in the large intestine and rectum they are decidedly rare. This fact renders any study of the histology of gastric polyps much more difficult. Secondly, the existence of intestinal metaplasia of the gastric mucosa is not well known and receives only a passing mention in text-books of pathology. Such a common and extensive change in an organ as important as the stomach deserves greater study.

The identification of the intestinal type of gastric polyp is based principally on two features. Firstly, it is essential that the adjacent mucous membrane shows complete intestinal metaplasia and that the epithelial tubules composing the polyp are in continuity with the metaplastic mucosa. Secondly. the tubules in the polyp must contain goblet cells. These can be demonstrated most vividly with mucicarmine, and are usually present in large numbers. However, if there is considerable loss of differentiation and marked epithelial hyperplasia the tumour shows fewer goblet cells. Of the five polyps composed of intestinal epithelium in this series all show many goblet cells except Case No. 2 in which there are rather few. This is explained by the loss of differentiation and widespread epithelial hyperplasia characteristic of malignant change.

Although the presence of a " striated " or " brush " border is a feature of epithelial cells of intestinal type, it has not been found very useful in the identification of the intestinal type of gastric polyp. The reasons for this are twofold. Firstly, the border of the cells tends to be obscured by the production of mucus within the cell and by the presence of excess mucus within the lumen of the tubules. And secondly, because the striated border can only be seen in particularly well-fixed material. Not all the polyps in this series came up to the high standard of fixation required. However, the striated border can be seen rather poorly in two of the intestinal types of gastric polyp but in none of those composed of true gastric mucosa. Paneth and argentaffin cells can nearly always be seen at the bases of the tubules in a mucosa showing intestinal metaplasia. In one of the intestinal types of gastric polyp (Case No. 1) they were present among the neoplastic cells lining the tubules in some numbers. However, their presence is not essential in the demonstration of the intestinal type of gastric polyp. Lastly, it must be emphasized that none of the gastric polyps composed of intestinal epithelium in this series contained more than a few tiny areas suggestive of true gastric epithelium. These served the purpose of indicating the origin of the tissue, for the sections might easily have been mistaken for tissue from the large or small intestine, if their origin from the stomach were not known.

In all the gastric polyps composed of intestinal epithelium in this series the surrounding mucous membrane shows complete intestinal metaplasia. It is justifiable to assume from this observation that the polyps arose from meta-

plastic mucosa. It is conceivable that polyps composed of true gastric epithelium may undergo intestinal metaplasia. To demonstrate this it is necessary to have both intestinal and true gastric epithelium in the same tumour; and for the surrounding mucous membrane to be predominantly of true gastric type. These conditions are not fulfilled in any of the twelve polyps in this series. However, there seems to be no reason why intestinal metaplasia should not occur in a polyp composed of true gastric epithelium.

Gastric polyps are uncommon. Seven out of the 12 investigated here were found in a consecutive series of 160 gastrectomy specimens removed for carcinoma (an incidence of 4·4 per cent). A control series of 80 gastrectomy specimens removed for benign gastric and duodenal ulcer contained no polyps. Stewart (1929) found that only 13 out of 263 surgical specimens of gastric carcinoma contained epithelial polyps (4·9 per cent). Miller, Eliason and Wright (1930) found an incidence of 4 per cent in their series of operation specimens. The relative rarity of gastric polyps is emphasized when their frequency is compared with that for intestinal and rectal polyps. Thus, Dukes (1926) found that 25 out of 33 consecutive operation specimens of rectal carcinoma contained one or more epithelial polyps (an incidence of 75 per cent). It is common to find small adenomas and papillomas in most surgical and post-mortem material from the large bowel, yet neither the surgeon nor the pathologist often come across epithelial polyps in the stomach.

The fact that malignant change may occur in benign epithelial tumours of the stomach is firmly established. Brunn and Pearl (1926) report that 12 per cent of their 84 examples of gastric polyps were malignant and Spriggs (1943) found that 9 out of 48 (or about 19 per cent) of his tumours show histological evidence of malignant change. In this series 4 out of 12 show the histological appearances of malignant change. Stewart (1929) states that the epithelial polyp in the stomach must definitely be accepted as a pre-cancerous lesion, but that the association is much less intimate than in the large intestine. It has been pointed out that epithelial polyps of the stomach are uncommon compared with their counterparts in the intestine and rectum. Their incidence in gastrectomy specimens removed for carcinoma is low, and according to Schindler (1950) they are only found in 2 per cent of persons undergoing gastroscopy. The incidence of polyps in the intestine and rectum in those undergoing sigmoidoscopy must be very considerably higher. It is probable, therefore, that benign epithelial tumours of the stomach account for only a small proportion of primary gastric carcinomata. They should be regarded as a definite, but rather uncommon pre-cancerous condition. Most carcinomas of the stomach probably arise as the result of a direct malignant change in the mucosa without the intermediate stage of benign adenomatous overgrowth.

A study of the histology of gastric polyps gives a clue to the histogenesis of gastric carcinoma. If some polyps in the stomach are composed of an intestinal type of epithelium then one would expect a certain proportion of gastric carcinomata to arise from this type of mucosa. Jarvi and Lauren (1951) have shown that gastric carcinomas may contain histological evidence of intestinal epithelium, even in their metastases. Mulligan and Rember (1954) report that 25 per cent of their series of gastric carcinomas are of the intestinal cell type. Evidence has been presented elsewhere which suggests that about 30 per cent of all gastric carcinomas arise from areas of intestinal metaplasia in the gastric

mucosa (Morson, 1955*b*). The fact that 5 out of the 12 gastric polyps in this series are composed of intestinal epithelium supports this conclusion. The evidence for the origin of carcinoma from areas of intestinal metaplasia was based on histological observations of the transition from metaplastic mucosa to carcinoma at the edge of the primary growth, on the study of early invading carcinomata, and on the appearances of carcinoma *in situ* in areas of intestinal metaplasia. In none of these examples was there any definite indication that benign adenomatous hyperplasia in metaplastic mucosa preceded the development of carcinoma.

It has been shown that there is more intestinal metaplasia of the gastric mucosa in cancerous than non-cancerous stomachs (Stout, 1945 ; Morson, 1955*a*) ; also, that a substantial proportion of gastric carcinomata arise from areas of intestinal metaplasia (Morson, 1955*b*). The demonstration that some gastric polyps are composed of intestinal epithelium is further support for this conclusion. This evidence also suggests that a stomach showing extensive intestinal metaplasia may be more prone to the development of carcinoma.

A number of reports in recent years have revealed that patients with pernicious anaemia are more prone to the development of carcinoma (Rigler and Kaplan, 1947 ; Mosbech and Videbaek, 1950). Rigler and Kaplan also suggest that the two diseases are probably linked through the medium of some common factor. It is known that the stomachs of persons with pernicious anaemia usually show very extensive intestinal metaplasia involving the fundus and body of the stomach, but the pylorus remains relatively normal (Magnus and Ungley, 1938). May not the reason for the increased incidence of gastric carcinoma in patients with pernicious anaemia be due to the extensive intestinal metaplasia characteristic of this disease? The distribution of intestinal metaplasia in pernicious anaemia may explain the unusual topographical distribution of primary carcinoma in the disease reported by Schell, Dockerty and Comfort (1954) and discussed elsewhere (Morson, 1955*b*). Further, if the stomachs of patients with pernicious anaemia are more prone than usual to cancerous change one would expect them to have a higher incidence of polyp formation. Brunn and Pearl (1943) found that 6 out of 43 patients with pernicious anaemia had gastric polyps, an incidence of nearly 14 per cent, which is about three times the incidence of polyps in gastrectomy specimens removed for carcinoma. Rigler and Kaplan (1947) report an incidence of 6·6 per cent, but others have also commented on the higher incidence of polyps in cases of pernicious anaemia. One would expect many of the gastric polyps found to be of the intestinal type in view of the extensive intestinal metaplasia of the gastric mucosa in this disease. One of the 12 polyps in this series was found in the pyloric part of the stomach removed for carcinoma from a patient with pernicious anaemia. Histologically, it is composed of true gastric epithelium. However, its presence in the pylorus may explain its histology, for this part of the stomach in pernicious anaemia is usually free of intestinal metaplasia, only the body and fundus being extensively affected. Schell, Dockerty and Comfort (1954) have studied the pathology of gastric carcinoma in 48 cases of pernicious anaemia and found benign mucosal polyps in 3 cases (6·3 per cent). They do not describe the histology of these, but they do report that all their cases showed " hyperplastic islands of intestinalization " at the fundus of the stomach which were large enough to suggest sessile polyps. In view of the observation that intestinal metaplasia of the gastric mucosa is uncommon at the fundus of the stomach

except in cases of pernicious anaemia (Morson, 1955*a*) it is significant that all of the cases described by Schell, Dockerty and Comfort (1954) should show what one may presume to be a pre-cancerous hyperplasia in metaplastic mucosa of intestinal type.

Accurate information on the histology of gastric polyps in pernicious anaemia must await the publication of a sufficiently large series of cases, but there is some evidence to suggest that the reason why patients with pernicious anaemia may be more prone to the development of gastric carcinoma and more susceptible to polyp formation in the stomach is because of the extensive intestinal metaplasia of the gastric mucosa associated with this disease.


## SUMMARY

1. A series of 12 gastric polyps have been examined. Five of these are composed of an intestinal type of epithelium. Three have been described and illustrated with microphotographs.

2. It is suggested that the intestinal type of gastric polyp arises from areas of intestinal metaplasia in the gastric mucosa.

3. That some gastric polyps are composed of intestinal epithelium, and may show evidence of malignant change, supports the conclusion made elsewhere that about 30 per cent of gastric carcinomas arise from areas of intestinal metaplasia in the gastric mucosa.

4. The relationship between gastric polyps, pernicious anaemia and carcinoma of the stomach has been briefly discussed.


I am indebted to the many surgeons who have supplied me with gastrectomy specimens; also to Mr. P. A. Runnicles and Mr. H. J. R. Bussey for the microphotographs. Expenses were provided out of a block grant from the British Empire Cancer Campaign.

## REFERENCES


Brunn, H. and Pearl, F.—(1926) *Surg. Gynec. Obstet.*, **48**, 559.—(1943) *Ibid.*, **76**, 257.

Dukes, C. E.—(1926) *Brit. J. Surg.*, **13**, 720.

Gleave, H. H.—(1923) *J. Path. Bact.*, **26**, 134.

Jacobson, W.—(1939) *Ibid.*, **49**, 1.

Jarvi, O. and Lauren, P.—(1951) *Acta path. microbiol. scand.*, **29**, 26.

Magnus, H. A.—(1937) *J. Path. Bact.*, **44**, 389.

*Idem* and Ungley, C. C.—(1938) *Lancet*, **1**, 420.

McManus, J. F. A.—(1948) *Amer. J. Path.*, **24**, 643.

Miller, T. B., Eliason, E. L. and Wright, V. W. M.—(1930) *Arch. intern. Med.*, **46**, 841.

Morson, B. C.—(1955*a*) *Brit. J. Cancer*, **9**, 365.—(1955*b*) *Ibid.*, **9**, 377.

Mosbech, J. and Videbaek, A.—(1950) *Brit. med. J.*, ii, 390.

Mulligan, R. M. and Rember, R. R.—(1954) *Arch. Path.*, **58**, 1.

Rigler, L. G. and Kaplan, H. S.—(1947) *J. nat. Cancer Inst.*, **7**, 327.

Schell, R. F., Dockerty, M. B. and Comfort, M. W.—(1954) *Surg. Gynec. Obstet.*, **98**, 710.

Schindler, R.—(1950) 'Gastroscopy.' Chicago (University of Chicago Press).

Spriggs, E. I.—(1943) *Quart. J. Med.*, **12**, 1.

Stewart, M. J.—(1913) *J. Path. Bact.*, **18**, 127.—(1929) *Brit. med. J.*, ii, 567.

Stout, A. P.—(1945) *N.Y. St. J. Med.*, **45**, 973.

Willis, R. A.—(1948) 'Pathology of Tumours.' London (Butterworth).

# OSTEOGENIC SARCOMA; AN ANALYSIS OF THE AGE AND SEX INCIDENCE

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THE purpose of this communication is to present an analysis of the age and sex distribution of a consecutive group of 67 osteogenic sarcomata, and to compare and supplement certain data with those obtainable from other published series. The cases here reported are derived from the records of the Bristol Bone Tumour Register (BTR), and represent all such tumours registered and classified during the period 1946–1954. Relevant details of individual cases are given in Table I.

It is not proposed in this paper to examine in any detail such aspects of osteogenic sarcoma as precise site of origin, histo-morphology, grading and prognosis, except where such may be germane to the general argument. It is, however, intended to present in the near future a further supplementary paper dealing with these several factors in full detail.

Firstly however, it is essential to emphasize that there are certain peculiar differences between bone sarcomata in juveniles and in older subjects. Hence it is expedient to sub-divide the material for analysis into three groups of cases according to age at time of onset:

1. Juvenile Group: Aged 0–34 years (31 cases—46 per cent).
2. Intermediate Group: Aged 35–49 years (7 cases—11 per cent).
3. Senile Group: Aged 50 years and over (29 cases—43 per cent).

The intermediate age group is less well defined than the other two, and relatively few in number. Thus it cannot profitably be considered in detail, and admits only the certain conclusion that bone sarcoma is rare during this middle period of life.

### *Anatomical Site Distribution of Present Series.*

This is indicated in Table II, and mainly confirms the findings of other well known and larger groups of cases.

### *Age Incidence of Complete Series.*

The crude age distribution curve of the total Bone Tumour Register (BTR) series is plotted in Fig. 1. The shape of this curve is characteristic of osteogenic sarcoma and shows a marked juvenile increase in the second decade, and also a smaller senile peak in the sixth decade. These features have given rise to the generally accepted mis-statement regarding the age of maximum incidence of osteogenic sarcoma. In the total number of cases seen it is true that the second decade produces the most; but when such total numbers are adjusted for population/age distribution it may then readily be seen that the incidence of this growth is high for the adolescent period, with a marked fall in the succeeding years. The

## Table I.

| No. | Initials. | Age. | Sex. | Site. | Type. | Histological Grade. | Concomitant skeletal disease. |
|---|---|---|---|---|---|---|---|
| 6 | K. S. | 18 | M. | Femur | B. | I | ——— |
| 10 | P. C. | 5 | M. | Humerus | B | II | ——— |
| 26 | N. G. | 13 | M. | ,, | B | I | ——— |
| 35 | N. J. | 14 | F. | Femur | B | I | ——— |
| 39 | S. H. | 19 | F. | ,, | B | I | ——— |
| 43 | D. C. | 21 | M. | ,, | B | I | ——— |
| 44 | F. P. | 42 | F. | Ilium | L | – | ——— |
| 61 | J. C. | 20 | M. | Tibia | B | II | ——— |
| 66 | R. D. | 19 | M. | ,, | B | III | ——— |
| 67 | G. C. | 67 | M. | Femur | L | II | Preceded by osteoclastoma. |
| 70 | A. L. | 58 | M. | Ilium | B | II | Advanced Paget's disease. |
| 81 | R. K. | 22 | M. | Humerus | B | II | ——— |
| 83 | G. J. | 73 | M. | Mandible | B | III | ——— |
| 94 | E. M. J. | 73 | F. | Tibia | B | III | Advanced Paget's disease. |
| 96 | F. T. | 38 | F. | Femur | B | II | ——— |
| 102 | F. A. H. | 70 | F. | ,, | B | II | ——— |
| 106 | J. W. | 27 | M. | Maxilla | L | III | Fibrous displasia. |
| 136 | Y. M. | 24 | F. | Femur | B | II | ——— |
| 143 | J. H. | 9 | M. | Vertebra | B. | II | ——— |
| 145 | K. W. | 26 | M. | Femur | B | II | ——— |
| 155 | A. G. | 71 | M. | Ilium | L | III | Advanced Paget's disease. |
| 157 | D. J. A. | 27 | M. | Femur | B | III | ——— |
| 163 | D. R. | 13 | M. | ,, | B | III | ——— |
| 169 | M. S. A. | 62 | F | Ilium | L | II | Early Paget's disease. |
| 191 | J. S. | 38 | M. | Femur | B | II | ——— |
| 198 | W. G. | 73 | M. | ,, | B | II | Advanced Paget's disease. |
| 203 | S. C. | 10 | F. | Humerus | B | I | ——— |
| 212 | R. M. | 62 | F. | Calvarium | L | III | Advanced Paget's disease. |
| 213 | E. C. | 16 | M. | Femur | B | II | ——— |
| 221 | M. C. | 14 | M. | ,, | B | II | ——— |
| 226 | A. O. | 64 | F. | Vertebra | B | II | ——— |
| 230 | C. H. | 86 | M. | Femur | L | III | ?preceded by enchondroma. |
| 250 | M. G. | 14 | M. | ,, | L | III | ——— |
| 273 | T. H. | 15 | M. | Humerus | B | III | ——— |
| 299 | F. B. | 67 | M. | ,, | L | II | Advanced Paget's disease. |
| 317 | C. P. | 89 | M. | Radius | L | I | Advanced Paget's disease. |
| 337 | J. B. | 36 | F. | Femur | L | II | ——— |
| 338 | A. D. | 60 | M. | Humerus | L | II | ——— |
| 406 | A. H. | 66 | M. | Femur | B | II | Advanced Paget's disease. |
| 414 | R. H. | 31 | M. | Humerus | B/C | II | ——— |
| 416 | W. M. | 69 | M. | Scapula | L | II | Advanced Paget's disease. |
| 423 | M. B. | 23 | F. | Femur | L | II | ——— |
| 428 | R. G. | 82 | F. | Femur | B | II | ——— |
| 440 | H. H. | 55 | F. | Femur | L | III | Advanced Paget's disease.. |
| 450 | J. B. | 60 | M. | Femur | L | II | ——— |
| 465 | D. M. | 49 | F. | Femur | L | II | ——— |
| 504 | E. H. | 51 | M. | Femur | L | II | Advanced Paget's disease. |
| 512 | A. H. | 76 | F. | Femur | L | II | ,, ,, ,, |
| 516 | J. R. | 75 | M. | Tibia | L | III | ,, ,, ,, |
| 522 | E. W. | 45 | F. | Femur | L | III | ——— |
| 529 | A. D. | 59 | F. | Femur | L | II | Advanced Paget's disease. |
| 538 | F. F. | 72 | M. | Ilium | B/C | II | ,, ,, ,, |
| 572 | A. R. | 80 | F. | Femur | L | II | ,, ,, ,, |
| 579 | J. C. | 20 | M. | Humerus | L | II | ——— |
| 586 | A. F. | 53 | F. | Tibia | L | II | Early Paget's disease. |
| 588 | E. M. | 59 | M. | Tibia | B/C | III | ——— |
| 590 | M. T. | 13 | M. | Femur | L | III | ——— |
| 592 | R. L. | 49 | M. | Femur | L | II | ——— |
| 595 | J. W. | 21 | F. | Rib | B | I | ——— |
| 664 | D. R. | 8 | M. | Femur | L | I | ——— |
| 675 | M. S. | 19 | M. | Humerus | L | II | ——— |
| 691 | D. M. | 18 | F | Femur | B | I | ——— |
| 705 | E. E. | 57 | F. | Femur | L | II | Advanced Paget's disease. |
| 737 | B. S. | 9 | F. | Femur | B/C | II | ——— |
| 741 | J. S. | 67 | F. | Tibia | L | II | ——— |
| 752 | A. M. | 10 | F. | Tibia | B | II | ——— |
| 754 | W. P. | 13 | F. | Humerus | B | II | ——— |

B = Osteoblastic Osteogenic Sarcoma.
L = Osteolytic Osteogenic Sarcoma.
B/C = Chondrogenic Osteogenic Sarcoma. A type with unusually large chondroid element in matrix.

*Note.*—Histological grading based upon author's method (Price, 1952).

TABLE II.

| *Anatomical Site.* | *Number.* | *Per cent.* |
|---|---|---|
| Femur | 35 | 52·2 |
| Humerus | 11 | 16·4 |
| Tibia | 8 | 11·9 |
| Ilium | 5 | 7·5 |
| Vertebra | 2 | 3·0 |
| Skull | 2 | 3·0 |
| Rib | 1 | 1·5 |
| Radius | 1 | 1·5 |
| Mandible | 1 | 1·5 |
| Scapula | 1 | 1·5 |

incidence then rises rapidly during the fifth decade, and over the whole life span reaches its maximum in the 7th and 8th decades (Fig. 2, 3 and 7).

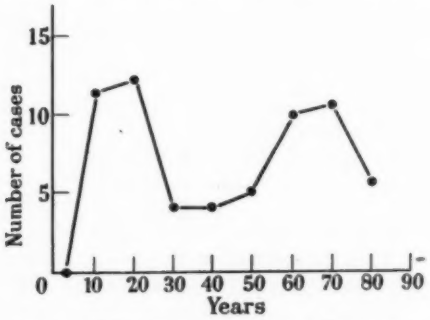


FIG. 1.—BTR Series. Crude age distribution.

It may immediately be suggested that the number of cases collected in the BTR records in the decade 15–24 years is unduly low, owing to the absence of young men on National Service with H.M. Forces during their 18th–20th years. A correction for this can be made by reference to Table A2 in the Registrar General's (1953) *Statistical Review of England and Wales* for the year 1952 (mid-year). The difference between the statistics given for the total home population and civilian populations (male) in the ages 15–24 years is 392,000, i.e. 14 per cent of all the males in this decade, or about 70 per cent of all males of age 18–20 years. This difference of course represents young men who are actually called up and posted. This figure has been checked by reference to the Regional Office of the Ministry of Labour and National Service which supplied the information that the proportion of those liable for national service who were either rejected or deferred was on average 34 per cent for men born in the years 1929–32. On correction therefore of the BTR series by plus 70 per cent of male sarcomata arising in the age period 18–20 years, a total relative tumour incidence figure of 1·72 is obtained for the second decade. This is still considerably lower than the comparable figures of 2·1 and 1·9 respectively for the 7th and 8th decades. This calculated figure (which is probably maximal) has been indicated as the separate point P in Fig. 2. In the case of women, the number actually involved in national service is too small to be of any significance.

In the BTR series, the tumour incidence in the later decades exceeds that of the 2nd, and the curve shows that in the 9th decade, the overall tumour incidence still remains high (Fig. 2). The corrected figures for age incidence of the BTR series, and for the further series of osteogenic sarcomata reported by the British Empire Cancer Campaing (1949), are separately plotted in Fig. 2, and it is interesting to note their close agreement.

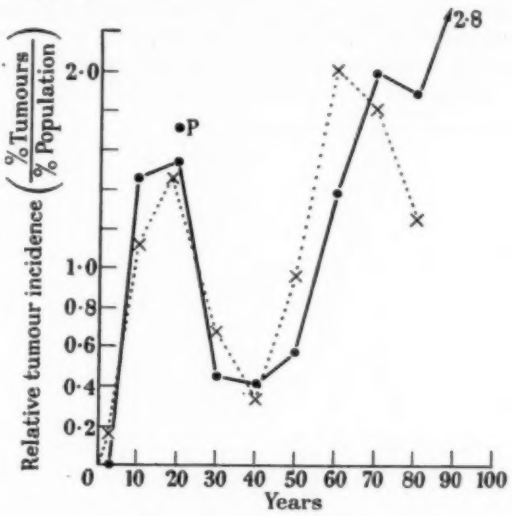


FIG. 2.—Corrected age incidence curves for :
BTR series of 67 osteogenic sarcomata. ——●——●——●
BECC (1949) series of 73 osteogenic sarcomata. ——×. . . .×. . . .×.
(All cases in each series, M + F.) The isolated point P indicates the calculated male figure, after making due allowance for men aged 18–20 years absent from the S.W. Region on National Service.

Fig. 3 shows the corrected age incidence curve for the combined BTR and British Empire Cancer Campaign (BECC) series of 140 sarcomata. This may be compared with a similarly corrected curve for the series of 308 osteogenic sarcomata published by Geschickter and Copeland (1949), in their Tables 17, 18, 21 and 22. The typical juvenile peak again appears in the American series, but the marked senile rise in incidence is absent.

The British series have been corrected for population age distribution by applying the 5 years' mean derived from Table A4, 1948–52, S.W. England and Greater London ; whilst the American series have been likewise adjusted by the mean 1930 and 1940 age distribution of the total population of the United States. (Table II, p. 872, *Vital Statistics Rates in the United States*, 1943). Comparison of these two sets of statistics shows that in the age period over 45 years there was a significantly higher proportion of persons in England and Wales than in the U.S.A., (9·2 per cent difference.) The respective figures given for proportion of total population in region over 45 years of age were :

*England and Wales*

S.W. region plus Greater London, (1948–52). 33·9%.

*United States of America*

Total population, (1930/40) . . . . . 24·7%

Due allowance has been made for this difference in calcuating the relative tumour incidence figures, both here and elsewhere. These figures are in each instance based upon the following factor :

$$\frac{\text{Per cent of tumours in series occurring in decade.}}{\text{Per cent of relevant population in same decade.}}$$

This is abbreviated in the tables to $\frac{\%T.}{\%P.}$

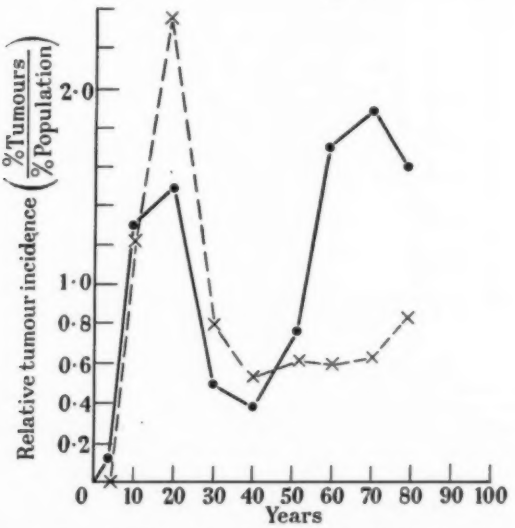


FIG. 3.—Corrected age incidence of combined group of 140 osteogenic sarcomata : (BTR 67 + BECC 73), ●——●——●.
Corrected age incidence of 308 osteogenic sarcomata : (From Tables 17, 18, 21 and 22, Geschickter and Copeland, 1949), ×- - - -×- - - -×.

This remarkable apparent difference in incidence of osteogenic sarcoma in the senior decades (Fig. 3) may in some part be due to the more complete differentiation of secondary chondrosarcoma as a tumour entity by Geschickter and Copeland (1949), who mention its predilection for patients of the age group 30–50 years, and assess its frequency as being about equal to that of primary chondrosarcoma. Moreover, they state that secondary chondrosarcoma is, in their experience, the commonest form of bone sarcoma complicating Paget's disease. In the BTR series, however, by far the most frequent variant seen in Paget's sarcoma has been the osteolytic osteogenic type—most often of spheroidal or spindle cell morphology. Even those histologists experienced in the diagnosis of bone sarcomata occasionally have difficulty in the differentiation of chondrosarcoma and certain osteogenic

sarcomata, in which there may be a high proportion of extracellular matrix. This is rendered still more uncertain by the typically broad spectrum of mesenchymal elements found in the latter tumour, and by indecision regarding the essentially chondroid or osteoid nature of the ill-formed stroma.

In support of the American series of Geschickter and Copeland (1949), entirely similar features obtain in the crude age distribution curve of Christensen (1925)—see Fig. 4, and in the tables published by Coley (1949). Both these latter series show the typical marked juvenile peak incidence in the 2nd decade, but the later big rise amongst older persons shown in the British series is absent. In the series of

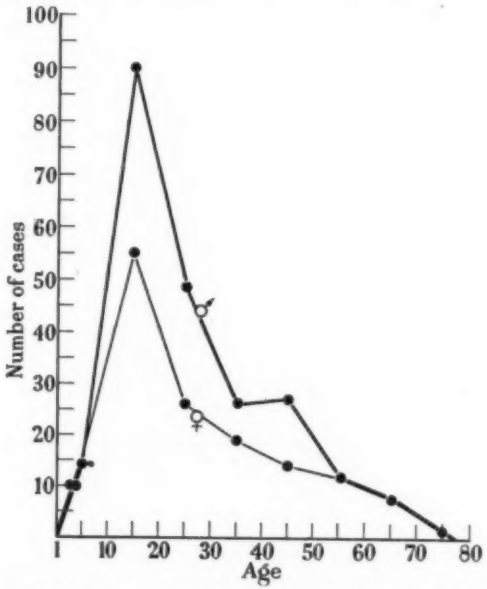


FIG. 4.—Age distribution of osteogenic sarcoma. (After Christensen, 1925.)
●——●——● Females, 150; ●——●——● Males, 226.

Geschickter and Copeland (1949), even after allowing for the difference in population age distribution, osteogenic sarcoma is still three times as common amongst young persons of age 5–24 years, as it is in the age group 55–74 years. In the British series (combined) this feature is reversed by a factor of 4 juvenile to 5 in the senile group.

It must be pointed out here that in any analysis of a series of cases of a given disease fallacies may arise due to the manner in which the material has been collected, and the available sources of such. The BTR series of 67 osteogenic sarcomata has been compiled upon a geographical rather than upon an institutional basis. The comparison of the numbers included in this with contemporary records, furnished by the Records Officer of the S.W. Region Cancer Records Bureau (Bristol) (1955, personal communication), indicates that the BTR cases represent about 75 per cent of the cases reported to him. Of the 67 patients 51 dwelt

in the Bath/Bristol area, whilst the remaining 16 were referred from Exeter (5), Salisbury (4), Bedford (2), Northampton (2), Gloucester, Coventry and Kent, 1 case each. The whole BTR group may therefore be regarded as being fairly representative of the incidence and distribution of this form of bone sarcoma amongst a well assorted sample of the population of the United Kingdom. By contrast, the BECC series of cases are derived from many sources, and are hence probably an entirely random sample, but devoid of any institutional bias.

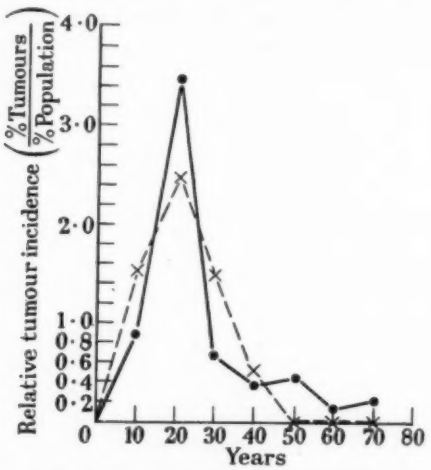


FIG. 5.—Corrected age incidence curve for group of 117 primary chondrosarcomata. (Geschickter and Copeland, 1949, Tables 9 and 10.)
Curve for 74 males, ●——●——●.
Curve for 43 females, ×- - - -×- - - -×.

Examination of the corrected age incidence curve for the closely allied tumour primary chondrosarcoma (data from Tables 9 and 10, Geschickter and Copeland, 1949), shows again a similar single peak of maximum age incidence in the 2nd decade (Fig. 5). Fig. 6 shows the corrected age incidence curves of two series of soft tissue fibrosarcomata. (British Empire Cancer Campaign, 1949 ; Stout, 1948). Also plotted in Fig. 6 is the corrected age incidence curve of one series of 97 lymphosarcomata (British Empire Cancer Campaign, 1949.) Broadly, these three curves all show an irregular but continued rise in sarcoma incidence with advancing age up to the 8th decade, with no sign of the well defined and distinct 2nd decade juvenile peak so characteristic of primary skeletal sarcomata.

From this comparison then one may conclude that in the causative mechanism of bone sarcoma there is a strongly acting aetiological factor which is present in the age period 5–24 years, and again in the British series throughout the later decades, although this is not confirmed by the American data.

*Analysis of the Sex Incidence*

The crude sex distribution figures of both the BTR and BECC series of osteogenic sarcomata are given in Tables III and IV.

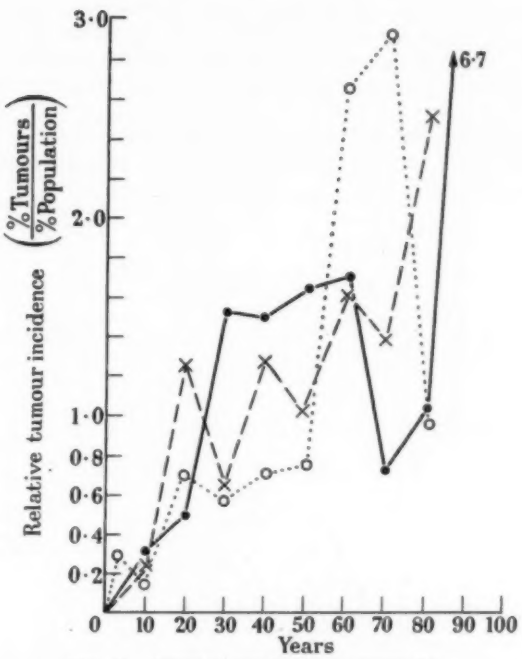


FIG. 6.—Corrected age incidence curves for:
Group of 200 fibrosarcomata (Stout, 1948), ●——●——●.
Group of 71 fibrosarcomata (BECC series, 1949), ×- - - -×- - - -×.
Group of 97 Lymphosarcomata (BECC series 1949), ○. . . .○. . . .○.

TABLE III.

| | Total | Males. | Females. |
|---|---|---|---|
| BTR series | 67 | 39 | 28 |
| BECC series | 73 | 41 | 32 |
| Combined Totals | 140 | 80(57%) | 60(43%) |

In a total of 308 osteogenic sarcomata, Geschickter and Copeland (1949) recorded 59 per cent males and 41 per cent females. The crude sex/age distribution of the combined British series are given in Table IV. Fig. 7 shows the relative

TABLE IV.

| | Years. | | | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0–. | 5–. | 15–. | 25–. | 35–. | 40–. | 45–. | 50–. | 55–. | 60–. | 65–. | 70–. | 75–. | 80–. | 85–. | |
| Male | 1 | 13 | 19* | 8 | 1 | 1 | 3 | 4 | 5 | 9 | 6 | 5 | 2 | 1 | 2 | 80 |
| Female | | 11 | 8 | 3 | 2 | 4 | 5 | 3 | 6 | 5 | 4 | 5 | 2 | 2 | | 60 |
| $f. = \frac{M.}{F.}$ | | 1·2 | 2·4 | 2·7 | 0·5 | 0·3 | 0·6 | 1·3 | 0·8 | 1·8 | 1·5 | 1·0 | 1·0 | 0·5 | | 1·3 |

* In view of the male absence on national service at age 18–20 years, the crude figure might be as high as 22 sarcomata, which would then give a sex factor $\left(\frac{M.}{F.}\right)$ of 2·8 in this decade.

tumour incidence for men and women after correction of the crude figures of Table IV for sex and age distribution of the home population.

From consideration of the combined British data, the following points appear:

(i) The juvenile female peak incidence occurs at a slightly earlier age than in the male (Fig. 7).

(ii) In both sexes alike, the incidence of osteogenic sarcoma is low during the intermediate age period, 30–45 years, when there also appears reversal of the otherwise usual male predominance.

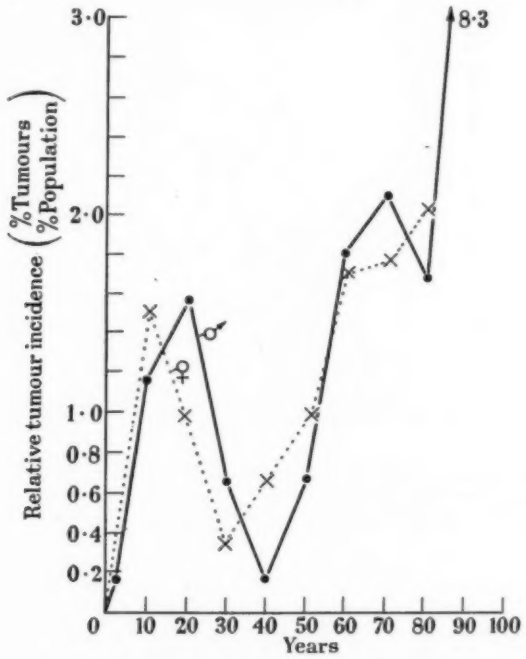


FIG. 7.—Relative incidence of osteogenic sarcoma.
Population corrected curves for: 80 males ——●——●——●. 60 females ——×. . . .×. . . .×. Combined BTR and BECC series of 140 cases.

(iii) After adjusting for differences in age and sex of the home population distribution, the figures shown in Table V are obtained for the corrected sex ratios.

(iv) Consideration of the juvenile age group (0–34 years) in greater detail in Table IV shows that in the combined BTR and BECC series of cases the female maximum tumour incidence appears in the age period 5–14 years, whilst in the male it occurs in the succeeding decade. This feature is absent on detailed analysis of the 213 sarcomata under 35 years of age recorded by Geschickter and Copeland (1949, Tables 17, 18, 21 and 22). On treating this American juvenile group as a complete series, both from the viewpoints of age and sex distribution, the following values were calculated for relative tumour incidence as set out in Table VI and Fig. 8.

TABLE V.

| | All Cases. | Age Groups. Juvenile. 0–34 | Intermediate. 35–49. | Senile. 50+. | |
|---|---|---|---|---|---|
| | 140 | 63 | 16 | 61 | |
| *Males :* | | | | | |
| Total sarcomata | 80 | 41 | 5 | 34 | |
| *Females :* | | | | | |
| Total sarcomata | 60 | 22 | 11 | 27 | |
| *Males :* | | | | | |
| % sarcomata in age group | 57 | 65 | 31 | 56 | = 100% |
| *Females :* | | | | | |
| Ditto | 43 | 35 | 69 | 44 | = 100% |
| *Males :* | | | | | |
| % Population in age group | 46·1 | 48 | 50·5 | 46 | = 100% |
| *Females :* | | | | | |
| Ditto | 53·9 | 52 | 49·5 | 54 | = 100% |
| *Tumour Incidence :* | | | | | |
| *Male* $\frac{\%\ T.}{\%\ P.}$ | 1·24 | 1·36 | 0·67 | 1·22 | |
| *Female* $\frac{\%\ T.}{\%\ P.}$ | 0·8 | 0·67 | 1·39 | 0·81 | |
| *Corrected Sex Factor :* | | | | | |
| $\frac{\text{T. Incidence Male}}{\text{T. Incidence Female}}$ | 1·55 | 2·0 | 0·48 | 1·54 | |

TABLE VI.

| | Age Group Under 5 yrs. | 5–14 yrs. | 15–24 yrs. | 25–34 yrs. |
|---|---|---|---|---|
| *Males :* | | | | |
| % Tumours in series | — | 21·8 | 59·7 | 18·5 |
| % Population in age group | 13·8 | 29·1 | 30·3 | 26·8 |
| *Tumour Incidence :* | | | | |
| Males $\frac{\%\ T.}{\%\ P.}$ | — | 0·75 | 1·96 | 0·69 |
| *Females :* | | | | |
| % Tumours in series | — | 32·5 | 55·1 | 12·7 |
| % Population in age group | 12·9 | 28·2 | 30·7 | 28·2 |
| *Tumour Incidence :* | | | | |
| Females $\frac{\%\ T.}{\%\ P.}$ | — | 1·15 | 1·79 | 0·45 |

In this table the high relative incidence figure for females of the age period 5–14 years is noteworthy.

*Discussion. Juvenile Group.*

The typical site of origin of osteogenic sarcoma in the young growing long bone is well known, and there are good reasons to relate this site frequency to an aberration of metaphyseal bone growth. Since this component contributes solely to length growth in long bones, it now becomes pertinent to inquire to what extent the known differences in skeletal development in the two sexes may be correlated with the following features :

1. The marked male preponderance of osteogenic sarcoma in juveniles.

2. The relatively earlier period of the female maximum tumour incidence, as shown by the combined British series of cases, and the greater relative incidence of this tumour in girls of age 5–14 years, as shown by both British and American data.

In this respect, total height of the individual can be taken as a useful index of skeletal length growth, the outstanding contribution to which is the growth which takes place at the lower end of the femur and upper end of the tibia. By combining the figures of differential epiphyseal growth in humans of Digby (1916), with those

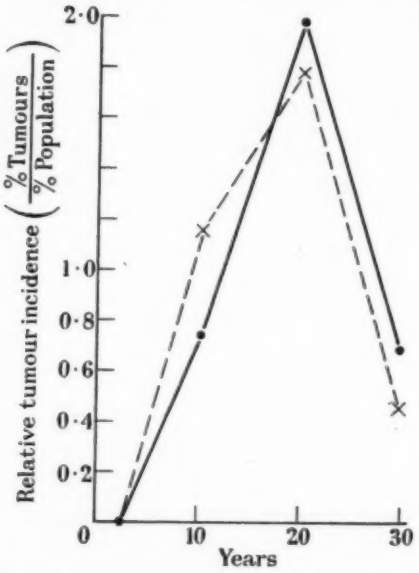


FIG. 8.—Corrected age incidence curve for group of 213 sarcomata (osteogenic). Males (124), ●——●——●. Females (89), ×- - - -×- - - -×. From Tables 17, 18, 21 and 22; Geschicketer and Copeland, (1949). All cases under 35 years of age.

stated for per cent contributions of femur and tibia to total height of the individual by Gill and Abbott (1942) it is possible to calculate that the distal femoral and proximal growth constitute respectively 20 and 12 per cent of the total height, out of an overall component of 51 per cent of height attributed to the leg.

In the human species, neither growth in height nor in weight progress evenly. It has long been known that child growth may be roughly divided in both sexes into three "springing-up" and three "filling-out" periods. These are depicted in the curves in Fig. 9, (after Stratz), and may be further given approximately in Table VII.

TABLE VII.—*"Springing-up" and "Filling-out" Periods in Males and Females.*

| | 1st. S.U.P. (yrs.) | 1st. F.O.P. (yrs.) | 2nd. S.U.P. (yrs.) | 2nd. F.O.P. (yrs.) | 3rd. S.U.P. (yrs.) | 3rd. F.O.P. (yrs) |
|---|---|---|---|---|---|---|
| Male | 1–2 | 3–5 | 6–7 | 8–12 | 13–15 | 16–20 |
| Female | 1st. | 2–5 | 6–7 | 7–9 | 9–12 | 13–20 |

On attempting to fit the springing-up periods of most active bone growth to the known incidence of osteogenic sarcoma it is unlikely that the 1st springing-up period has much bearing, in view of the extreme rarity of bone sarcoma under the age of 5 years (0·7 per cent of present combined series).

Complete data are unfortunately scanty, but in the four years following the 2nd springing-up period (i.e. age 7–10 years, inclusive), in the combined figures of the BTR series and those of Geschickter and Copeland (1949), there appears in males 6·3 per cent, and in females 12 per cent of a total of 242 osteogenic sarcomata under 35 years of age. At this period the difference between male and female populations is under 1 per cent, both for Britain and the U.S.A. The total number of cases is very small, but here, again, the reversed sex incidence agrees with that

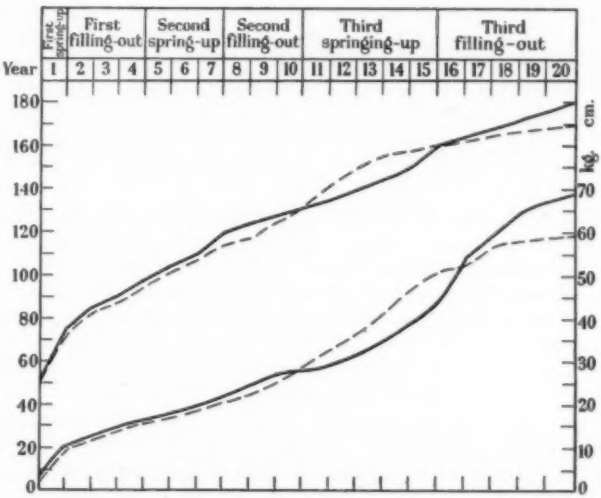
First spring-up
First filling-out
Second spring-up
Second filling-out
Third springing-up
Third filling-out
Year
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
180 160 140 120 100 80 60 40 20 0
cm.
kg.
70 60 50 40 30 20 10 0

FIG. 9.—The growth curve according to Stratz, Boys———; Girls– – –, from 'Bone Growth in Health and Disease', by H. A. Harris. (p. 90).

noted above for the wider age period of 5–14 years ,(Table VII). This suggests that even at this very young age the difference in tumour incidence between the sexes is due to the relatively advanced bone growth in girls, which has been estimated to be, on average, 1 year in front of boys at chronological age 9 years (Todd, 1937). This difference in bone age is thought to increase to as much as 2 or 3 years on attaining chronological age 12 years. It should however be added that the 1st and 2nd growth spurts are not so well defined generally, or in the individual, as the 3rd—the so-called " adolescent growth spurt ".

This adolescent acceleration of bone length growth is characteristic of man and primates, and is said not to take place in other mammals. Its earlier onset in girls is now well established as occurring at age 9–12 years, the data reported by Simmons and Greulich (1943) and Simmons (1944) confirming the earlier findings of Shuttleworth (1938). In boys this adolescent growth spurt takes place at 12–14 years of age. These figures agree well with the position of maximum gradient in the

curves shown in Fig. 9. The data published by Schwerz (1928), quoted by Gill and Abbott (1942), also shows that this increased rate of growth in girls precedes that of boys for the individual long bone (e.g. femur and tibia) by 1 to 2 years, but with some quantitative variation between different bones.

The data compiled by various workers have indicated that, in girls, the time of onset of the adolescent growth spurt is closely related to the time of appearance of the menarche and onset of puberty ; furthermore, it has been noted by Stone and Barker (1937) that the hypofeminoid type of girl has a late menarche and delayed adolescent growth spurt, although this latter may be eventually prolonged to lead to greater than average height, so resembling more closely the male growth pattern. It is clear, of course, both in Europeans and Americans, that the adolescent growth spurt of boys, though later than that of girls by about 2 to 3 years, on average produces greater male height. In both sexes, however, this measurement is influenced also by parental stature (Harris *et al.*, 1930).

In the light of these anthropometric findings, then, it is here suggested that the earlier increased relative incidence of osteogenic sarcoma in young girls is due to the earlier age of occurrence of the adolescent growth spurt. The greater total tumour incidence in the young male is due to the relatively increased length growth of the skeleton, i.e. both time and tissue bulk factors being unduly weighted in favour of a greater male " risk ".

This latter explanation is strongly supported by the high sarcoma incidence in the lower end of the femur, where length growth is maximal, and its relative rarity in the short tubular bones of hand and foot. Whilst the latter grow in length from a single terminal epiphyseal plate, the absolute linear growth in centimetres for each bone is much less than that of the femur shaft and other major appendicular long bones.

### *The Senile Age Group (Over 50 Years).*

*Age distribution*—all cases in combined BTR and BECC series (Table IV).

Although the uncorrected age distribution curve in Fig. 1 shows maximum tumour incidence in the 7th decade, the corrected age incidence curve for the BTR series indicates that there may possibly be a further rise in the age period 85–94 years. This feature would resemble the general tread of fibrosarcoma (Fig. 6), but the number of cases encountered at this advanced age is too small to permit any definite conclusion upon this point.

*Incidence of Paget's osteitis deformans.*

The separate and combined totals of cases associated with Paget's disease of bone are shown in Table VIII.

TABLE VIII.—*Osteogenic Sarcomata associated with Paget's disease.*

| | Males. | | | | | Females. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 50–. | 60–. | 70–. | 80–. | Total. | 50–. | 60–. | 70–. | 80–. | Total. |
| BTR | 2 | 3 | 4 | 1 | 10 | 4 | 2 | 2 | 1 | 9 |
| BECC | | | | | 6 | | | | | 7 |
| Combined | | | | | 16 | | | | | 16 |
| Incidence in total cases in age group (34) | | | | | 47% | Incidence in total cases in age group (27) | | | | 59% |
| Incidence in combined series of 61 sarcomata (over 50 yrs.) | | | | | | | | | | 52·4% |

This table shows the approximately equal sex distribution of Paget's disease in cases of osteogenic sarcoma over 50 years of age, the condition being well advanced and active in about one half of the senile bone sarcomata encountered. On correcting these crude figures for the sex distribution of persons over 50 years (Males 46 per cent, Females 54 per cent), the totals in Table VIII would be modified to give : males, 16 sarcomata ; females, 14 sarcomata. There is again here a small relative male preponderance.

During examination of the BTR collection of specimens, evidence has been found suggesting extra-osseous connective tissue changes in Paget's disease. In one case (BTR/510, male aged 52) well advanced Paget's disease was associated with a soft tissue parosteal fibrosarcoma of the leg. Furthermore, although in the BTR juvenile group osteoblastic osteogenic sarcoma was seen three times as often as the osteolytic form, in the senile group this factor is reversed, the latter tumour being about three times as common.

Site analysis of the BTR series of sarcomata associated with Paget's disease fully confirms the point emphasized by Davie and Cooke (1937) that such growths are not infrequently multicentric in origin, and arise in the bone damaged by the osteitic process. Therefore, in general terms, the anatomical site distribution of the senile form of this sarcoma runs largely parallel with the skeletal distribution of Paget's disease, i.e. related to bone which is of membranous or periosteal origin, and with a relatively high site frequency in flat bones.

*Senile osteogenic sarcoma not related to Paget's disease.*

Two features have been noted in these cases :

A. Very occasionally there is evidence which suggests that the sarcoma is secondary to a pre-existing benign tumour, e.g. BTR/67, G. C.— male, aged 67 ; giant cell tumour of lower end of femur. BTR/230, C. H— male, aged 86 ; enchondroma of diaphysis of femur.

B. In some instances, particularly in femoral lesions, there has been radiographic and histological evidence of advanced osteoporosis. Histologically, as age advances, bone structure becomes more clearly defined, and both analytical and histochemical methods have shown tissue dehydration and relative loss of the mucopolysaccharide component of cartilage and inter-fibre cement substance. Histological examination of bone structure in the immediate vicinity of a number of senile osteogenic sarcomata, however, suggests that the continuation of this osteoporotic process may eventually lead to very extensive bone destruction via demineralization and disorganisation of the organic framework. Consequent vicarious formation of much new osteoid is also seen.

*Sex distribution.*

The sex distribution and relative tumour incidence for the combined BTR and BECC cases over 50 years of age are given in Table IX.

Examination of the combined BTR and BECC figures for cases over 50 years of age shows a crude sex ratio, (M/F.), of f. = 1·26, i.e. 5 males to 4 females. On correction, however, for population sex distribution, this ratio increases to f. = 1·54 i.e. 3 males to 2 females. This factor then approaches that found in the juvenile group, where f. = 2·0 (Table V). This male preponderance appears possibly to be reversed in the age period 50–60 years (f. = 0·75), and to be lower still in the preceding intermediate age group of 35–49 years, where f. = 0·48 (Table X).

TABLE IX.

| | Age. | | | | |
|---|---|---|---|---|---|
| *Males.* | 50–. | 60–. | 70–. | 80–. | Total. |
| Total tumours | 9 | 15 | 7 | 3 | 34 |
| % Tumours | 26 | 44 | 21 | 9 | 56 |
| % Population in age group | 44·8 | 33·4 | 17·6 | 4·2 | 46 |
| Tumour incidence $\frac{\%\ T.}{\%\ P.}$ | 0·6 | 1·3 | 1·2 | 2·1 | 1·22 |
| *Females.* | | | | | |
| Total tumours | 9 | 9 | 7 | 2 | 27 |
| % Tumours | 33 | 33 | 26 | 8 | 44 |
| % Population in age group | 40·8 | 32·9 | 20·5 | 5·8 | 54 |
| Tumour incidence $\frac{\%\ T.}{\%\ P.}$ | 0·8 | 1·0 | 1·26 | 1·4 | 0·8 |
| *Sex Factor*, corrected to f $= \frac{\text{T. incidence, M.}}{\text{T. incidence, F.}}$ | 0·75 | 1·3 | 0·95 | 1·5 | 1·54 |

Note.—Per cent of tumours in each decade calculated upon basis of total tumours, (M. or F.) over 50 years = 100.

Per cent of population in each decade based upon proportions of male and female population in decade, of all persons over 50 years of age.

Totals in last column calculated from ratio :

$$\frac{\text{Per cent tumours male (or female) over 50 years.}}{\text{Per cent population male (or female) over 50 years.}}$$

TABLE X.—*Intermediate Age Group—35–49 years.*

| | Intermediate Age Group—35–49 years. |
|---|---|
| *Males.* | |
| Total tumours in period | 5 |
| % Total tumours in period | 31·2% |
| % Total population in age period | 50·5% |
| Tumour incidence. $\frac{\%\ T.}{\%\ P.}$ | 0·67 |
| *Females.* | |
| Total Tumours in period | 11 |
| % Total tumours in period | 68·8% |
| % Total population in age period | 49·5% |
| Tumour incidence. $\frac{\%\ T.}{\%\ P.}$ | 1·4 |
| *Corrected Sex Factor.* | |
| f. $= \frac{\text{T. incidence, male.}}{\text{T. incidence, female.}}$ | 0·48 |

The inference from these figures is that the factor or factors which cause the relative male preponderance of osteogenic sarcomata in juveniles, are still operative in the senile group. It has already been suggested that this male frequency is related to the overall greater bone length growth of men, which feature may also basically influence the increased male incidence of Paget's disease in like manner. The data of Table VIII would support this assertion, but are not conclusive, since even after correcting for the population sex distribution at this age, the male relative increase is only very small. As in the juvenile group, so here also, there is some suggestion from the corrected age/sex distribution curve (Fig. 7) that the incidence of osteogenic sarcoma in later years rises somewhat more rapidly for women.

*Discussion—senile group of cases.*

On comparison, the sex incidence differences noted in senile cases of osteogenic sarcomata are absent in the groups of fibrosarcomata and lymphosarcomata of similar age of the several series considered above. In the BECC 1949 series of both of these latter tumours, the corrected sex factor (M./F) in respect of patients over 45 years of age was found to be 0·85 for lymphosarcoma and 1·05 for fibrosarcoma. In the larger American series of fibrosarcomata of Stout (1948) no details are given for the age/sex distribution ; it was however stated that the total group included 100 men and 106 women.

(*Note.*—Although lymphosarcoma usually shows a well marked male preponderance when all ages are combined, the BECC 1949 series records 34 males and 31 females over 45 years of age. On applying to these figures the appropriate correction for sex distribution in the population over 45 years, the ratio

$$\frac{\text{Tumour incidence, male}}{\text{Tumour incidence, female}} = 0{\cdot}85.)$$

These differences suggest that assuming the causative mechanism of these three forms of sarcoma to be of a similar nature, in the case of osteogenic sarcoma the relative frequency in persons over 50 years of age is markedly influenced by some added factor which is sex hormone dependant.

## SUMMARY AND CONCLUSIONS.

1. A series of 67 osteogenic sarcomata has been analysed for the factors of age and sex distribution and incidence. The resulting data have been supplemented by a further series of 73 osteogenic sarcomata reported by the British Empire Cancer Campaign (1949). These figures have been compared and contrasted with two American series, and also with groups of cases of primary chondrosarcoma, lymphosarcoma and fibrosarcoma.

2. The data presented shows that the incidence of osteogenic sarcoma is relatively higher amongst the population of England (S.W. Region) during the 7th and 8th decades than in the 2nd decade, as is most generally believed. In view of the small number of cases available in this series it is not clear as to the relative incidence in the 9th decade, although there is some suggestion that this may be still higher than in the two preceding periods. These latter features resemble the late age incidence trends in soft tissue fibrosarcoma. This latter tumour however does not show, in the series examined, the differential male preference which is seen in osteogenic sarcoma, both juvenile and senile.

3. The sex incidence of the cases of the age period under 35 years has been correlated with the known features of juvenile bone growth. It is suggested that the sex incidence differences are due to the characteristically earlier development of the female skeleton, and the greater total bone growth in length of the male.

4. The intermediate age group (35–49 years) provides too few cases for detailed treatment, but indicates the comparative rarity of bone sarcoma of this type during this period of life. In the very small number of cases considered there is reversal of the otherwise usual male predominance.

5. In the senile group (over 50 years) one half of all cases here reported were associated with Paget's osteitis deformans. After correction for differences in population age/sex distribution the male sex predominance is again found to be about the same order as that encountered in the juvenile group.

In addition to the original list of cases formerly studied by the author (1952), further material has been included here from the following sources, all being cases referred to the Bristol Bone Tumour Register :

| | |
|---|---|
| J. Bastow, Esq., F.R.C.S. | Nos. 516, 705. |
| Dr. S. Curwen, D.M.R.T. | No. 67. |
| A. L. Eyre-Brook, Esq., F.R.C.S. | Nos. 44, 450, 512, 754. |
| Dr. H. J. Gibson. | Nos. 522, 572. |
| Dr. R. C. Hadden. | Nos. 406, 414. |
| Dr. E. J. Harries. | No. 595. |
| H. K. Lucas, Esq., F.R.C.S. Ed. | Nos. 416, 423, 440. |
| Dr. E. M. Martland | No. 675. |
| W. R. Mitchell, Esq., F.R.C.S. | Nos. 691, 752. |
| J. A. Penrose, Esq., F.R.C.S. | No. 664. |
| K. H. Pridie, Esq., F.R.C.S. | Nos. 504, 538. |
| Dr. R. Sandry. | Nos. 737, 741. |
| J. B. Shield, Esq., F.R.C.S. | Nos. 579, 590, 592. |
| Dr. G. Stewart Smith. | No. 586. |
| Dr. A. L. Taylor | Nos. 428, 529. |
| Dr. J. C. Valentine. | Nos. 465, 588. |

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Fig. 4 is reproduced by kind consent of the author and publishers of the *Annals of Surgery* ; Fig. 9 has been copied by permission of the publishers. This courtesy is also acknowledged with thanks.

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## REFERENCES.

British Empire Cancer Campaign.—(1949) *Rep. Brit. Emp. Cancer Campgn*, **27**, 289.

Christensen, F. C.—(1925) *Ann. Surg.*, **81**, 1074.

Coley, B. L.—(1949) ' Neoplasms of Bone.' New York (Hueber).

Davie, T. B. and Cooke, W. E.—(1937) *Brit. J. Surg.*, **25**, 299.

Digby, K. H.—(1916) *J. Anat., London*, **50**, 187.

Geschickter, C. F. and Copeland, M. M.—(1949) ' Tumours of Bone.' 3rd. Ed. London (Lippincott).

Gill, G. G. and Abbott, Le R. C.—(1942) *Arch. Surg.*, **45**, 286.

Harris, J. A., Jackson, C. M., Paterson, D. G. and Scammon, R. E.—(1930) ' The Measurement of Man.' Minneapolis (Univ. Minnesota Press).

Price, C. H. G.—(1952) *Brit. J. Cancer*, **6**, 46.

Registrar General.—(1953) *Statistical Review of England and Wales, for the year* 1952, Tables, Part II, Civil. London (H.M. Stationery Office).

Simmons, K.—(1944) Monogr. Soc. Res. Ch. Dev. 9. No. 1.

*Idem* and Greulich, W. W.—(1943) *J. Pediat.*, **22**, 518.

Shuttleworth, F. K.—(1938) Monogr. Soc. Res. Ch. Dev. 3. No. 5.

Stone, C. P. and Barker, R. G.—(1937) *Hum. Biol.*, **9**, 1.

Stout, A. P.—(1948) *Cancer*, **1**, 30.

Stratz, .—(1933) Quoted in " Bone Growth in Health and Disease " by H. A. Harris, p. 90. London (Oxford University Press).

Todd, T. W.—(1937) ' Atlas of Skeletal Maturation. St. Louis. (Mosby).

*Vital Statistics Rates in the United States*, 1900–1940—(1943) Edited by Linder, F. E. and Grove, R. D. Washington, D.C. (U.S.G.P.O.).

# A DERMATOMYOSITIC SYMPTOM-COMPLEX ASSOCIATED WITH MALIGNANT DISEASE.

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It was Unverreicht who in 1891 gave the name of " dermatomyositis " to an acute illnesss characterised by fever, dermatitis, oedema and polymyositis. Subsequent publications included many instances of the chronic progressive type which Petges and Clejat (1906) rightly separated out under the title of " Sclerose atrophique de la peau et myosite generalisee "—a variant of progressive systemic sclerosis of which scleroderma is a cutaneous manifestation and not a synonym. It was not until 1935 that Bezecny first noted the association of a relatively acute form of dermatomyositis with coincidental neoplasm. He recorded then two such instances—one fatal case with carcinomata of the breasts and one with ovarian carcinomata whose skin and muscle lesions remitted after surgical treatment. The relevant literature since that time contains nearly seventy similar examples—sufficient to make it evident that the association is not merely fortuitous. A study of other published series suggests that more—and more thorough—post mortem examinations would have disclosed many more examples. But all these authors have been content to accept these as true cases of dermatomyositis, failing to differentiate this symptom-complex from the Petges-Clejat type or from the acute fulminating polymyositic variant of polyarteritis nodosa.

Three cases of this association are briefly recorded.

*Case No.* 1.

Mr. C. O—, aged 56, was admitted to a Service Hospital on November 1, 1952. He had been in excellent health until about four months previously when he began to notice increasing breathlessness, easy fatigue and a dry cough which gradually became constant. Six weeks before admission, following a day of sun exposure while sailing, a bright red rash had appeared over his nose and cheeks and had quickly spread to appear on the dorsa of his hands and feet and at the bases of his fingernails. In the next month, he had noticed the onset of stiffness and pain on movement of most of the limb joints and general weakness and exhaustion became more evident. In the week prior to admission he became fevered, developed a pleuritic pain below the right scapular angle and became very dyspnoeic, with both ankles very swollen. Examination showed a plump distressed patient who had lost weight, with temperature 102° F., auricular fibrillation and congestive cardiac failure. A dusky erythematous rash was present in the " butterfly " distribution over his nose and cheeks, his brow, the dorsal surfaces of his forearms, hands and the terminal phalanges of his fingers. There was some tenderness in, and pain on movement of, the limb muscles, especially of the upper arms and thighs, but oedema was limited to both ankles and to the sacral area. There was dullness of

percussion at both lung bases and a loud pleural friction rub posteriorly below the right scapula. Investigations showed Hb 70%; W.B.C. 8600/cmm.; E.S.R. 54 mm./1 hour; L.E. cell test, negative; plasma proteins total 5·6 G per cent (albumen 2·7, globulin 2·9, A/G ratio 0·9/1); urine, a trace of albumen; x-ray chest, generalised cardiac enlargement and consolidation of right lower lobe. Skin biopsy showed follicular hyperkeratosis with oedema and perivascular round cell infiltrate and some areas of collagen degeneration in the corium. A tentative diagnosis of systemic lupus erythematosus with pleuropneumonia and congestive cardiac failure was made and considerable immediate improvement followed on treatment with digoxin and chloramphenicol. Within the next two weeks, muscular weakness became much more evident, together with moderate tenderness and pain on movement of muscles so that he became unable to raise his arms or legs from the bed. The rash became more florid and was accentuated by pitting oedema of the underlying tissues round the orbits and much more so on the limbs. The diagnosis of acute dermatomyositis was then made and seemed to be confirmed when in a few days dysphagia with nasal regurgitation of fluids appeared. His general condition deteriorated extremely rapidly thereafter and though treatment with cortisone was commenced he died on 28.xi.52.

Post-mortem findings were of a bronchogenic carcinoma of the right lung with metastases to the mediastinal glands and gross dilatation of the heart chambers. Macroscopically there was a peculiar pinkish pallor of the muscles and sections, including myocardial ones, showed in varying degree:

(*a*) Areas of degeneration and disintegration with patchy loss of striation, some empty sarcolemmar sheaths, some containing retraction bands and a great increase in sarcolemma nuclei.

(*b*) Focal lymphocytic infiltration in the interstitial connective tissue which appeared to be increased in some parts.

There was no evidence of vascular change in the muscle sections. Examination of other tissue and organ sections showed debatable eosinophilic collagen change in some portal tracts and renal connective tissue, adrenal capsule, oesophageal submucosa and pancreatic connective tissue. Arterioles in the adrenal medulla and pancreas also showed some eosinophilic hyaline change in their walls.

*Case No.* 2.

W. G—, aged 59, an engine driver, first attended the Skin Out-patient Department in October, 1949, with a non-specific, pruritic, indurated erythema of his brow, his neck and the dorsa of his hands. This condition waxed and waned over the next three months, but in January, 1950, it became acutely flared up and was accompanied then by the onset of a generalised crippling weakness of his muscles, starting in the upper arms and thighs, but quickly involving his shoulders and lumbar muscles so that he became quite helpless. On admission to hospital in February, 1950, there was also elicited a history of increasing dyspnoea over the previous three to four months—a symptom latterly difficult to separate from the general strain of attempted muscular exertion—and of vague inconstant left-sided pleuritic pain. There had been no noticeable weight loss. Examination confirmed the presence of an acute oedematous rash and of great muscular weakness, without significant wasting but with slight tenderness and a soft boggy doughiness of the larger muscle masses and marked diminution of the tendon reflexes and electrical

reactions. Physical examination otherwise revealed only dullness and crepitations at both bases posteriorly. Investigations showed a mild hypochromic anaemia ; W.B.C. total 7500 cm.; E.S.R. 90mm./1 hour ; plasma proteins, normal ; urine, creatine 585 mg./24 hours and 17-ketosteroids 8·7 mg./24 hours ; x-ray chest, bullous emphysema at both apices ; increased markings at both bases, especially on the left side ; E.C.G., normal record ; bronchoscopy, negative ; skin biopsy, non-inflammatory changes ; muscle biopsy (the surgeon reported absence of fasciculation on contact with scalpel), focal degenerative changes with oedema and cellular infiltrate in the interstitial connective tissue.

Treatment of the dermatomyositis was attempted with testosterone propionate vitamin E, but gradual deterioration followed with progressive emaciation and muscle atrophy with loss of tendon reflexes, increasing dyspnoea and a chronic cough latterly productive of foul blood-stained sputum. A left-sided pleural effusion developed, paracentesis producing blood-stained fluid.

Before his death on August 4, 1950, the rash had begun to fade to an atrophic telangiectatic thickening on the affected sites.

Post-mortem findings were those of a carcinoma of the left lower lobe bronchus with widespread metastases to lymph nodes and to the medulla of the right adrenal gland—histologically an anaplastic large spheroidal cell carcinoma. The rash was still evident and some palpably indurated areas of skin were noted and the affected muscles of the limbs, abdominal wall and diaphragm showed an odd pallor and lack of firmness in consistency with conspicuously adherent perimuscular fascia.

Skin sections showed focal hyperkeratosis and follicular plugging, thinning of the epidermis and flattening of the rete pegs, while in the dermis perivascular round cell infiltration and areas of collagen degeneration could be seen. Muscle sections showed focal disintegration and degenerative changes, an increase of interstitial connective tissue and a few lymphorrhages. Sections of other organs and tissues showed basophilic collagen degeneration of connective tissue in some places, while a few vessels, particularly arterioles, showed eosinophilic fibrinoid swelling of the intima.

*Case No.* 3.

J. M—, aged 80—a retired crofter—was admitted to hospital on July 11, 1950. His health had been very good until six months previously, when there had developed a bright red, irritating rash on his cheeks and nose. This had gradually spread to the brow, chin and neck, to the dorsa of his hands and wrists and the extensor surface of his forearms. On the face there had been considerable puffiness, especially round his eyes. Following the appearance of the rash he began to notice increasing general fatigue and weakness, which had slowly progressed so that he could now barely lift his arms or raise his feet from the ground. For several months he had indigestion with frequent vomiting after food and, shortly before admission, swallowing had become extremely difficult and fluids had tended to regurgitate through his nose. Examination showed an emaciated old man with a confluent red oedematous rash on the exposed parts of his face, hands and forearms. His muscles were indurated and tender and painful on movement and extremely, weak with a moderate amount of wasting. A palpably enlarged hard supraclavicular lymph node was present on the left side. His pulse was irregular with frequent extrasystoles. Investigations showed : Hb. 65 per cent.; W.B.C. 11,000/c.mm.;

E.S.R. 25 mm./1 hour; plasma proteins, normal; urinary creatine, 345 mg./24 hours; E.C.G., biphasic T wave in leads ii and iii indicating myocarditis; Ba. swallow impossible—choking with spilling of barium into the trachea; skin biopsy, epidermal inflammatory changes present; the corium showed hyaline degeneration of collagen and dilated vessels with a perivascular round cell infiltrate; muscle sections showed patchy degenerative changes.

Treatment with P.A.B.A. produced no improvement and muscular weakness and wasting progressed with loss of tendon reflexes. He became progressively more emaciated and at his own request in August, 1950, he returned home, where he shortly died. No post-mortem examination was carried out.

A study of these and other published records reveals several interesting features:

(*a*) Unlike adult acanthosis nigricans which is usually associated with intra-abdominal cancer, this apparent "dermatomyositis" may be found with neoplasm, of any organ or tissue. Reports include tumours of breast (Behrman and Forman, 1939), the ovary (Brunner and Lobraico, 1951) the uterus (Duverne, Bonnayme and Mounier, 1954), the larynx (Borda, 1953), the lung (Quiroga and Bottrick, 1950), the bronchus (Bouton, Mendoza and Ravera, 1953), the oesophagus (Jorgensen, 1944), the stomach (Lipman and Tober, 1950), the intestine (McCombs and MacMahon, 1947), the rectum (O'Leary and Waisman, 1940), the gall bladder (Wolf and Wilens, 1936), and the kidney (Cottell, 1952). More unusual reports include Hodgkin's disease (Curtis, Blaylock and Harrell, 1952) and other reticuloses (Sheldon, Young and Dyke, 1939), plasmocytoma (Jorgensen, 1944), multiple myeloma, retroperitoneal sarcoma and a tumour of the parotid (Curtis, Blaylock and Harrell, 1952). O'Leary (1949) quotes also two instances of Cushing's syndrome suggestive of adrenal tumour and Sunde (1949) has recently recorded a case in a girl of twelve with a chromophobe adenoma of the hypophysis. The presence of metastases is frequently, but not constantly recorded.

(*b*) There seems to be no doubt that in all instances the neoplastic process has preceded the onset of cutaneous and muscular changes though the recognition of the latter has very often preceded the detection of the former, which may only be discovered post mortem. The dermatomyositic process appears also undoubtedy to hasten the end.

(*c*) In several instances (Brunner and Lobraico, 1951; Curtis Blaylock and Harrell, 1952; Duverne, Bonnayme and Mounier, 1954), Bezecny's (1935) observation of remission with successful treatment of the neoplasm has been confirmed permanently or temporarily with relapse (Curtis, Blaylock and Harrell, 1952; Dostrovsky and Sagher, 1946), Lipman and Tober (1950), on the appearance of metastases.

(*d*) Though several authors have recorded cases of association of neoplasm with other collagen diseases such as scleroderma (Curtis, Blaylock and Harrell, 1952; Lipman and Tober, 1950) and systemic lupus erythematosus (Lansbury, 1953) the reports available do not exclude a diagnosis of "dermatomyositis" of this type. In my opinion, it is just this association with neoplasm which separates this symptom-complex from dermatomyositis of the Petges-Clejat type and from progressive systemic sclerosis.

From the clinical aspect the main distinguishing features appear to be the onset in the relevant age-group of a relatively acute illness characterised by erythematous skin lesions which very closely, in appearance, distribution and even histology,

mimic those of systemic lupus erythematosus but for the very marked oedema which is localised to the sites of the rash. Though it may have been wisdom after the event, in Case 1 exposure to strong sunlight had briefly preceded the appearance of the rash. In prolonged cases, as in Case 2, the rash may settle to a more chronic pattern of poikiloderma. Muscle involvement which appears to be more of the shoulder and pelvic girdles and the proximal rather than the distal limb muscles advances from moderate swelling with tenderness and pain on movement to an atrophic paresis. It may be significant that tendon reflexes disappear—perhaps an indication of nerve involvement—and there are recorded examples of diminished peripheral sensation (Berg, 1951). Involvement of the myocardium qua muscle appears to be found frequently as in Case No. 3 of this series. Any other visceral lesions (pleurisy, ascites, etc.) are attributable to the neoplasm or to metastases. The finding of albuminuria or haematuria, for example, would invite relevant investigation for a renal or visceral growth. Unless the course of the illness is altered by successful treatment of the neoplasm, death follows quickly.

Biopsy and post-mortem sections of skin and muscle show non-specific changes of an inflammatory nature in the former and of focal disintegration or degeneration in the latter. Wainger and Lever (1949) describe connective tissue damage in the myocardium which appeared to increase the degeneration of muscle fibres. Some of the visceral sections of Cases 1 and 2 do suggest a more general involvement of connective tissue and the walls of smaller blood vessels in a widespread process of fibrinoid degeneration type. Reports by others stress particularly the vascular lesions found post mortem. McCombs and MacMahon (1947) found widespread lesions varying from " acute necrosis and chronic sclerosing oedema of all layers to healed lesions with intimal and adventitial scarring ".

Theories on the basic factors of this association can only be speculative. Biopsy and post-mortem reports have shown nothing to suggest a mechanical dissemination of tumour cells in the skin and muscles. Consideration of comparable recorded entities does provide a possible line of elucidation of this problem. A number of authors have described various forms of nervous degeneration in association with malignant disease which is most commonly but not always of bronchogenic type. Denny-Brown (1948) has published cases of this type with sensory neuropathy and myopathy which he has claimed to be due to primary simple degeneration of dorsal root ganglion cells associated with a primary degeneration of muscles. He presumed the cause to be a metabolic disorder related to the tumour cells, the changes being similar to changes seen in animals in pantothenic acid and vitamin E deficiency states. Henson (1953) has described eight such cases with a lower motor neurone atrophic paresis affecting mainly proximal muscle groups, though some showed a pseudobulbar palsy with dysphagia and dyarthria and others ptosis or diplopia. Hart (1954) has recorded two similar instances—one with a carcinoma of the breast and the other of the antrum—a male patient who incidentally showed poikilodermatous patches on the face, neck and arms. Both these improved greatly with treatment of the neoplasm though credit was given to the administration of vitamin B complex—especially pyridoxine—and a hypothesis of competition by tumour cells for this vitamin was postulated. One must assert that the skin lesions in this dermatomyositic illness are not those of frank pellagra. Lennox and Pritchard (1950) also described a series of cases, some of whom showed the proximal motor neuropathy without significant sensory change. Histological examination is such cases has shown patchy demyelination in the cord and

peripheral nerves. I believe that there may well be a close relationship between these neuropathies and this type of "dermatomyositis" and would suggest histopathological examination of post-mortem cord and nerve specimens in any future cases.

It is tempting to suggest therefore that the pathogenesis of this dermatomyositic complex lies in the growth factor or invasive potential of the neoplastic process—that by competing with normal connective tissue or muscle or nerve cells for some essential nutritional factor, or perhaps by flooding the circulation with an enzymatic spreading factor like hyaluronidase to assist invasion it might provoke widespread alteration and degeneration of connective tissue ground substance, and related mesenchymal derivatives such as muscle or nerve cells. Alternatively an antigenic substance from the tumour or its breakdown products might first sensitise and then provoke an hyperimmune response, particularly in the smaller blood vessels, with secondary degenerative effects on connective tissue muscles and nerves.

The importance of awareness and recognition of this dermatomyositic symptom-complex lies in the obviously vital necessity in these cases for an exhaustive search for a neoplasm which may still be amenable to operative or radiation therapy.

SUMMARY.

(1) These examples are presented of the association of a dermatomyositic symptom-complex with malignant disease.

(2) It is suggested that this symptom complex which is an entity separate from true chronic progressive dermatomyositis may be closely related to the published cases of nervous degeneration in association with malignant disease and, like these, may originate from a competition by tumour cells for some essential nutritional factor.

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## REFERENCES.

BEHRMAN, S. AND FORMAN, L.—(1939) *Proc. Roy. Soc. Med.*, **32**, 1575.
BERG, R. L.—(1951) *New Engl. J. Med.*, **245**, 822.
BEZECNY, R.—(1935) *Arch. Derm. Syph., Wien*, **171**, 242.
BORDA, J. M.—(1953) *Rev. Asoc. méd. argent.*, **67**, 755.
BOUTON, J., MENDOZA, D. AND RAVERA, J. J.—(1953) *Torax*, **2**, 47.
BRUNNER, M. J. AND LOBRAICO, R.—(1951) *Ann. intern. Med.*, **34**, 1269.
COTTELL, C. E.—(1952) *Amer. J. med. Sci.*, **224**, 160.
CURTIS, A. C., BLAYLOCK, H. C. AND HARRELL, E. R., Jr.—(1952) *J. Amer. med. Ass.*, **150**, 844.
DENNY-BROWN, D.—(1948) *J. Neurol. Psychiat.*, **11**, 73.
DOSTROVSKY, A. AND SAGHER, F.—(1946) *Brit. J. Derm.*, **58**, 52.
DUVERNE, J., BONNAYME, R. AND MOUNIER, R.—(1954) *J. Méd., Lyons*, **35**, 601.
HART, P. L. DE V.—(1954) *Brit. med. J.*, i, 606.
HENSON, R. A.—(1953) *Proc. Roy. Soc. Med.*, **46**, 859.
JORGENSEN, K. S.—(1944) *Acta. Path. microbiol., scand.*, **21**, fasc iv.
LANSBURY, J.—(1953) *Ann. Rheum. Dis.*, **12**, 301.
LENNOX, B. AND PRITCHARD, S.—(1950) *Quart. J. Med.*, **19**, 97.
LIPMAN, M. P. AND TOBER, J. N.—(1950) *Gastroenterology*, **16**, 188.

McCombs, R. P. and MacMahon, H. E.—(1947) *Med. Clin. N. Amer.*, **31**, 1148.
O'Leary, P. A.—(1949) *Ibid.*, **33**, 28.
*Idem* and Waisman, M.—(1940) *Arch. Derm. Syph., Chicago*, **41**, 1001.
Petges, G. and Clejat, C.—(1906) *Ann. Derm. Syph., Paris*, **7**, 550.
Quiroga, M. I. and Bottrick, A.—(1950) *J. Amer. med. Ass.* (*Abstracts*), **142**, 1028.
Sheldon, J. H., Young, F. and Dyke, S. C.—(1939) *Lancet*, i, 82.
Sunde, H.—(1949) *Acta paediatr., Stockh.*, **37**, 287.
Unverreicht, H.—(1891) *Dtsch. med. Wschr.*, **17**, 41.
Wainger, C. L. and Lever, W. F.—(1949) *Arch. Derm. Syph., Chicago*, **59**, 196.
Wolf, A. and Wilens, S. L.—(1936) *Amer. J. Path.*, **12**, 239.

# ALTERATION IN GROWTH OF EHRLICH MOUSE ASCITES CARCINOMA ON REPEATED TRANSFER THROUGH HAMSTERS.

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THE Ehrlich mouse ascites cancer can be transferred to hamsters, where it can grow with preservation of its character of ascites cancer (Ahlström and Ising, 1955). By transplantation at 3-day intervals the cancer can be transferred from hamster to hamster and survive several passages. The purpose of the present investigation was to determine whether the ascites cancer undergoes any change during successive passages through a long series of hamsters.

## MATERIAL AND METHODS.

The Ehrlich mouse ascites carcinoma was originally obtained from Dr. Georg Klein, Institute for Cell Research, Stockholm. All mice used belonged to the same inbred strain.

The experimental animals were Syrian golden hamsters weighing 100–120 g. All of the animals were brought up on a standard diet.

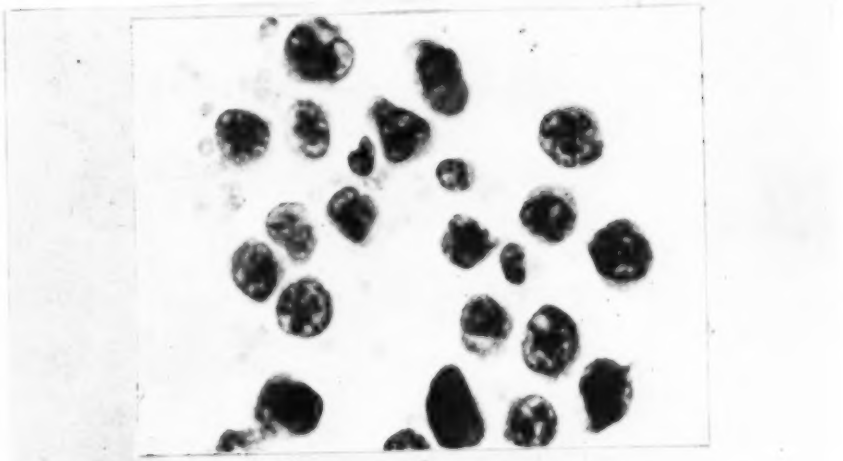
The first hamster in Series I was inoculated intraperitoneally with 5 ml. of pooled ascitic fluid from a batch of mice inoculated 6 days before. The hamster was killed 3 days after the inoculation. The ascitic fluid was carefully collected, measured and, after determination of its content of cancer cells, transferred to another hamster. The cancer was then transmitted in the same way at 3-day intervals to new hamsters until, after a number of passages, the cancer gave the impression that it was about to die. The ascitic fluid was then injected intraperitoneally into a few mice. The mice were killed as soon as they showed signs of ascites, and the cancer was again transferred to a hamster. It was then again carried through a series of hamsters (Series II) until the number of cancer cells in the ascitic fluid was so small that there was a risk of the cancer dying. The cancer was resuscitated by a new passage through mice, after which a new series of hamsters was started (Series III). In this way the cancer was passed through 7 series of hamsters, each series consisting of 6 to 9 passages. The last hamster series (Series VIII) was interrupted after 44 passages, since it now appeared that the ascites cancer could be transferred *ad infinitum*.

Smears were taken from each animal and stained according to Papanicolaou. The cancer cells were counted in a Buerker's haemocytometer, counting 9 squares

## EXPLANATION OF PLATES.

FIG. 1.—Ascites cancer cells during successive passages in Series II. A: the donor mouse. B: the first hamster passage. C: the eighth hamster passage. Papanicolaou staining. × 880.

FIG. 2.—Ascites cancer cells during the successive passages in Series VIII. A: the donor mouse. B: the twenty-fifth hamster passage. C: the forty-fourth hamster passage. Papanicolaou staining. × 880.



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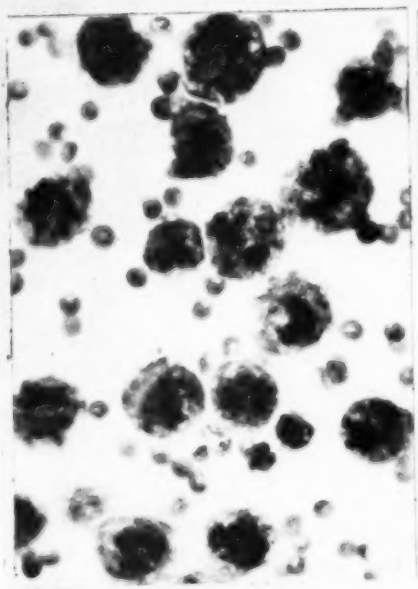

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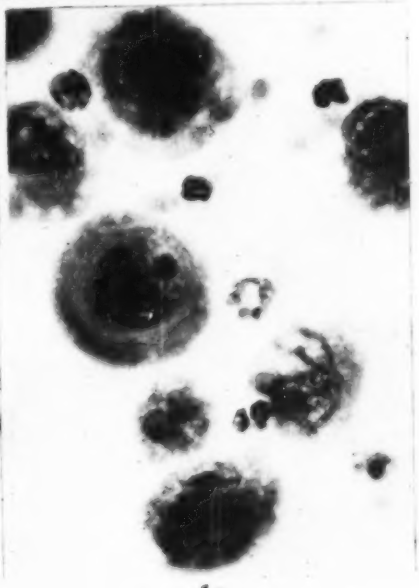

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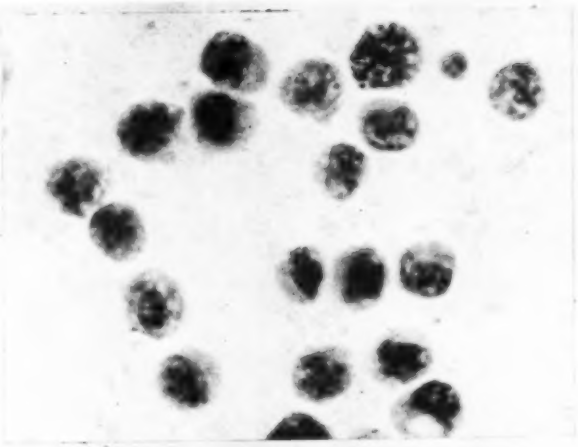

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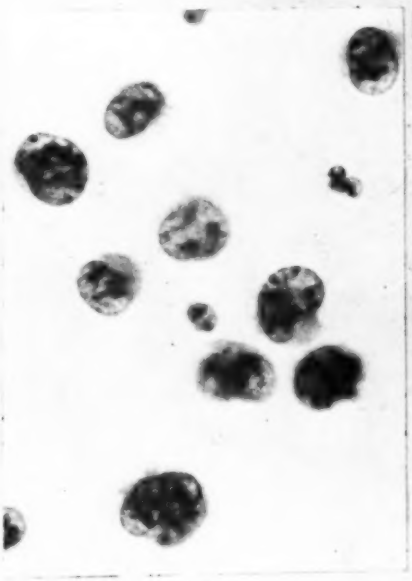

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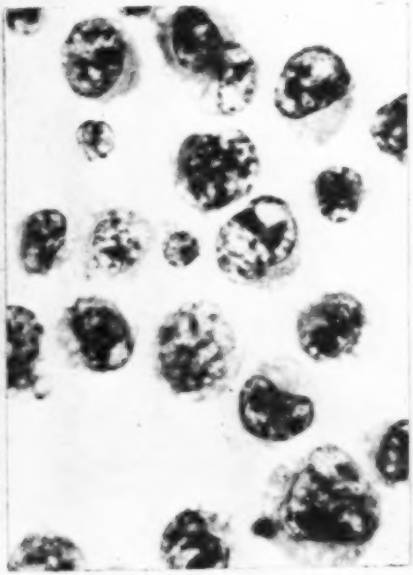

2c

of each chamber (each square containing 0·1 mm³.). The sample of the ascitic fluid to be used for cell counting was diluted 1 : 100 or 1 : 200 with physiological saline containing 0·05 per cent eosin.

The number of non-tumourous cells in the ascitic fluid was assessed by the number found in the smears : 1000–3000 neoplastic and non-neoplastic cells were counted ; the number of leukocytes and mononuclear cells was correlated with 100 cancer cells.

The number of cancer cells in mitosis per 100 cancer cells was taken as the mitotic index. The number of cancer cells counted ranged from 1500 to 3000.

## RESULTS.

### I. *General Course of the Experiments.*

The results are given below in Table I and Fig. 3. The left half of the table contains data about the ascitic fluid each animal received. The right half of the

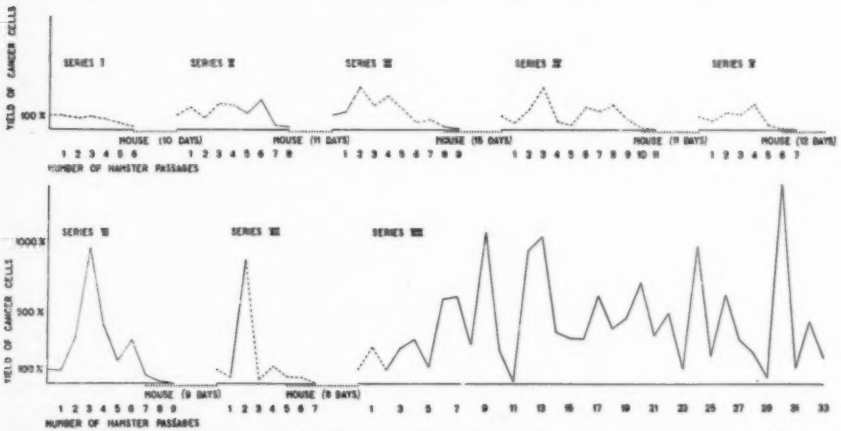
YIELD OF CANCER CELLS
100 %
SERIES I
SERIES II
SERIES III
SERIES IV
SERIES V
MOUSE (10 DAYS)
MOUSE (11 DAYS)
MOUSE (15 DAYS)
MOUSE (9 DAYS)
MOUSE (12 DAYS)
1 2 3 4 5 6
1 2 3 4 5 6 7 8
1 2 3 4 5 6 7 8 9
1 2 3 4 5 6 7 8 9 10 11
1 2 3 4 5 6 7
NUMBER OF HAMSTER PASSAGES
YIELD OF CANCER CELLS
1000 %
500 %
100 %
SERIES VI
SERIES VII
SERIES VIII
MOUSE (9 DAYS)
MOUSE (8 DAYS)
1 2 3 4 5 6 7 8 9
1 2 3 4 5 6 7
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33
NUMBER OF HAMSTER PASSAGES

FIG. 3.—The yield of cancer cells in 8 consecutive series of hamster passages. The yield of cancer cells in each passage hamster was calculated as a percentage of the number of cancer cells injected 3 days before.

table gives information on the ascitic fluid at the death of the hamster 3 days later. The yield of cancer cells in each hamster, calculated as a percentage of the number injected, provides a measure of growth of the cancer cells and is given in the last column but one of the table. The diagram in Fig. 3 is based on the figures in this column. The continuous lines in the diagram denote that the host was a female hamster ; the dotted lines, that the host was a male hamster.

In the first 7 series the cancer survived only 5–10 passages. It is possible that in Series I and II the cancer cells might have been able to survive still another passage. The poorest growth of the cancer cells was seen in Series I, where the yield of cancer cells from each hamster never exceeded the amount injected. On two occasions in Series III and IV the number of cells increased to threefold the number injected. In two instances in Series VI and VII the increase was almost ten-fold.

TABLE I.—*Transmission of Ehrlich Mouse Ascites Carcinoma through 8 Consecutive Series of Hamsters.*

The left half of the table contains data on the ascitic fluid each hamster received; the right half gives information on the ascitic fluid when the animal was killed.

| Passage. | Volume of inoculated ascitic fluid in ml. | Number of cancer cells $\times 10^6$ per ml. | Total number of inoculated cancer cells $\times 10^6$ | Volume of ascitic fluid after 3 days in ml. | Number of cancer cells $\times 10^6$ per ml. | Total number of cancer cells $\times 10^6$ after 3 days. | Yield of cancer cells per cent. | Mitotic index. |
|---|---|---|---|---|---|---|---|---|
| Series I: | | | | | | | | |
| 1 | 5·0 | 56·4 | 282·0 | 2·25 | 122·0 | 275·0 | 98 | 1·22 |
| 2 | 2·25 | 122·0 | 274·0 | 2·75 | 81·4 | 224·0 | 82 | 1·56 |
| 3 | 2·50 | 81·4 | 204·0 | 3·50 | 55·2 | 193·0 | 94 | 1·81 |
| 4 | 2·50 | 55·2 | 138·0 | 3·10 | 33·0 | 102·0 | 74 | 1·12 |
| 5 | 3·0 | 33·0 | 99·0 | 2·00 | 23·3 | 46·6 | 47 | 2·85 |
| 6 | 2·0 | 23·3 | 46·6 | 0·30 | 31·8 | 9·5 | 20 | 2·00 |
| | | | | ↓ mouse for 10 days | | | | 2·14 |
| Series II: | | | | | | | | |
| 1 | 2·5 | 104·9 | 262·3 | 9·5 | 38·6 | 366·7 | 155 | 1·34 |
| 2 | 2·5 | 38·6 | 96·5 | 1 | 81·2 | 81·2 | 85 | 1·39 |
| 3 | 1·0 | 81·2 | 81·2 | 1·75 | 84·6 | 148·1 | 182 | 1·84 |
| 4 | 1·6 | 84·6 | 135·4 | 2·4 | 98·2 | 235·7 | 174 | 0·71 |
| 5 | 2·4 | 98·2 | 235·7 | 10·2 | 26·6 | 271·3 | 115 | 0·48 |
| 6 | 5·0 | 26·6 | 133·0 | 2·4 | 116·6 | 279·8 | 210 | 1·36 |
| 7 | 2·3 | 116·6 | 268·2 | 2·2 | 38·9 | 85·6 | 32 | 0·97 |
| 8 | 2·2 | 38·9 | 85·6 | 0·5 | 32·3 | 16·2 | 19 | 1·84 |
| | | | | ↓ mouse for 11 days | | | | 1·28 |
| Series III: | | | | | | | | |
| 1 | 3·0 | 112·5 | 337·5 | 6·7 | 60·9 | 408·0 | 121 | 0·45 |
| 2 | 2·0 | 60·9 | 121·8 | 4·8 | 76·8 | 368·6 | 303 | 0·30 |
| 3 | 2·0 | 76·8 | 153·6 | 3·7 | 70·6 | 261·2 | 170 | 0·95 |
| 4 | 2·0 | 70·6 | 141·2 | 1·7 | 199·5 | 339·2 | 240 | 1·40 |
| 5 | 1·7 | 199·5 | 339·2 | 5·6 | 84·3 | 472·1 | 148 | 0·60 |
| 6 | 2·0 | 84·3 | 168·6 | 1·2 | 79·7 | 95·6 | 56·6 | 0·90 |
| 7 | 1·0 | 79·7 | 79·7 | 0·7 | 27·6 | 19·3 | 70 | 1·20 |
| 8 | 0·7 | 27·6 | 19·3 | 0·1 | 39·3 | 3·9 | 20 | 3·20 |
| 9 | 0·1 | 39·3 | 3·9 | 0·2 | — | — | — | — |
| | | | | ↓ mouse for 15 days | | | | 1·85 |
| Series IV: | | | | | | | | |
| 1 | 2·0 | 62·9 | 125·8 | 0·4 | 161·9 | 64·8 | 51·5 | 1·00 |
| 2 | 0·4 | 161·9 | 64·8 | 1·8 | 51·4 | 92·5 | 143 | 1·40 |
| 3 | 1·8 | 51·4 | 92·5 | 3·0 | 92·6 | 277·8 | 300 | 1·70 |
| 4 | 2·0 | 92·6 | 185·2 | 2·4 | 47·4 | 113·8 | 61 | 1·20 |
| 5 | 2·0 | 47·4 | 94·8 | 0·8 | 42·4 | 33·9 | 36 | 2·20 |
| 6 | 0·8 | 42·4 | 33·9 | 0·9 | 59·9 | 53·9 | 159 | 1·20 |
| 7 | 0·9 | 59·9 | 53·9 | 0·8 | 91·4 | 73·1 | 135 | 0·80 |
| 8 | 0·8 | 91·4 | 73·1 | 0·7 | 183·9 | 128·7 | 176 | 1·20 |
| 9 | 0·7 | 183·9 | 128·7 | 0·8 | 119·3 | 95·4 | 74 | 1·60 |
| 10 | 0·8 | 119·3 | 95·4 | 1·3 | 5·1 | 6·6 | 7 | 2·80 |
| 11 | 1·0 | 5·1 | 5·1 | 0·05 | — | — | — | — |
| | | | | ↓ mouse for 11 days | | | | 2·0 |
| Series V: | | | | | | | | |
| 1 | 2·0 | 103·0 | 206·0 | 0·8 | 167·3 | 133·8 | 65 | 1·4 |
| 2 | 0·8 | 167·3 | 133·8 | 1·0 | 165·3 | 165·3 | 124 | 1·0 |
| 3 | 1·0 | 165·3 | 165·3 | 3·1 | 58·9 | 187·6 | 113 | 2·6 |
| 4 | 2·0 | 58·9 | 117·8 | 1·6 | 135·0 | 216·0 | 184 | 1·9 |
| 5 | 1·6 | 135·6 | 216·0 | 1·8 | 46·6 | 83·9 | 39 | 2·5 |
| 6 | 1·8 | 46·6 | 83·9 | 0·3 | 13·9 | 4·2 | 5 | 2·8 |
| 7 | 0·2 | 13·9 | 2·78 | — | — | — | — | — |

TABLE I—*cont.*

| Passage. | Volume of inoculated ascitic fluid in ml. | Number of cancer cells × $10^6$ per ml. | Total number of inoculated cancer cells × $10^6$ | Volume of ascitic fluid after 3 days in ml. | Number of cancer cells × $10^6$ per ml. | Total number of cancer cells × $10^6$ after 3 days. | Yield of cancer cells per cent. | Mitotic index. |
|---|---|---|---|---|---|---|---|---|
| | | | | ↓ | | | | |
| | | | | mouse for 12 days | | | | 1·9 |
| Series VI: | | | | | | | | |
| 1 | 2·0 | 251·6 | 503·2 | 6·5 | 75·6 | 491·4 | 97 | 2·6 |
| 2 | 2·0 | 75·6 | 151·2 | 14·7 | 33·5 | 492·5 | 326 | 2·0 |
| 3 | 2·0 | 33·5 | 67·0 | 7·0 | 92·0 | 644 | 961 | 0·9 |
| 4 | 2·0 | 92·0 | 184·0 | 12·6 | 60·3 | 759·8 | 413 | 1·0 |
| 5 | 2·0 | 60·3 | 120·6 | 5·3 | 38·5 | 204·1 | 167 | 0·5 |
| 6 | 2·0 | 38·5 | 77·0 | 5·5 | 44·4 | 244·2 | 317 | 1·4 |
| 7 | 2·0 | 44·4 | 88·8 | 6·0 | 8·9 | 53·4 | 60 | 1·2 |
| 8 | 2·0 | 8·9 | 17·8 | 5·3 | 0·5 | 2·7 | 14 | 3·8 |
| 9 | 2·0 | 0·5 | 1·0 | — | — | — | — | — |
| | | | | ↓ | | | | |
| | | | | mouse for 9 days | | | | 1·7 |
| Series VII: | | | | | | | | |
| 1 | 2·0 | 115·5 | 231·0 | 2·9 | 37·3 | 108·7 | 47 | 1·0 |
| 2 | 2·0 | 37·3 | 74·6 | 6·7 | 97·9 | 656·8 | 880 | 3·2 |
| 3 | 2·0 | 97·9 | 195·8 | 2·9 | 21·5 | 62·4 | 32 | 2·9 |
| 4 | 2·0 | 21·5 | 43·0 | 1·0 | 53·9 | 53·9 | 125 | 4·1 |
| 5 | 1·0 | 53·9 | 53·9 | 0·5 | 52·8 | 26·4 | 49 | 4·1 |
| 6 | 0·5 | 52·8 | 26·4 | 0·3 | 38·4 | 11·5 | 44 | 3·0 |
| 7 | 0·3 | 38·4 | 11·5 | — | — | — | — | — |
| | | | | ↓ | | | | |
| | | | | mouse for 8 days | | | | 1·9 |
| Series VIII: | | | | | | | | |
| 1 | 2·0 | 47·8 | 95·6 | 2·5 | 99·1 | 247·8 | 259 | 1·4 |
| 2 | 1·5 | 99·1 | 148·7 | 1·0 | 161·5 | 161·5 | 102 | 1·2 |
| 3 | 1·0 | 161·5 | 161·5 | 8·5 | 48·5 | 412·3 | 255 | 1·0 |
| 4 | 2·0 | 48·5 | 97·0 | 2·4 | 124·9 | 299·8 | 309 | 0·9 |
| 5 | 2·0 | 124·9 | 249·8 | 6·5 | 45·9 | 298·4 | 119 | 1·7 |
| 6 | 2·0 | 45·9 | 91·8 | 10·2 | 53·9 | 549·8 | 599 | 0·9 |
| 7 | 2·0 | 53·9 | 107·8 | 16·2 | 40·8 | 661·0 | 613 | — |
| 8 | 2·0 | 40·8 | 81·6 | 16·0 | 14·0 | 224·0 | 274 | 0·4 |
| 9 | 2·0 | 14·0 | 28·0 | 8·5 | 35·4 | 300·9 | 1075 | — |
| 10 | 2·0 | 35·4 | 70·8 | 4·0 | 42·8 | 171·2 | 242 | — |
| 11 | 2·0 | 42·8 | 85·6 | 2·0 | 1·0 | 2·0 | 2 | — |
| 12 | 2·0 | 1·0 | 2·0 | 1·5 | 12·5 | 18·8 | 940 | — |
| 13 | 1·2 | 12·5 | 15·0 | 1·8 | 86·9 | 156·4 | 1043 | — |
| 14 | 1·5 | 86·9 | 130·4 | 9·2 | 52·0 | 478.4 | 367 | — |
| 15 | 2·0 | 52·0 | 104·0 | 10·7 | 31·1 | 332·8 | 320 | 1·2 |
| 16 | 2·0 | 31·0 | 62·2 | 3·0 | 65·9 | 197·7 | 318 | — |
| 17 | 2·0 | 65·9 | 131·8 | 9·5 | 86·3 | 819·9 | 622 | — |
| 18 | 2·0 | 86·3 | 172·6 | 6·0 | 112·4 | 674·4 | 391 | — |
| 19 | 2·0 | 112·4 | 224·8 | 10·2 | 102·6 | 1046·5 | 465 | — |
| 20 | 2·0 | 102·6 | 205·2 | 15·5 | 95·1 | 1474·1 | 718 | 1·0 |
| 21 | 2·0 | 95·1 | 190·2 | 8·0 | 81·0 | 648·0 | 341 | — |
| 22 | 2·0 | 81·0 | 162·0 | 5·3 | 152·3 | 807·2 | 498 | 2·1 |
| 23 | 2·0 | 152·3 | 304·6 | 5·0 | 68·3 | 341·5 | 112 | — |
| 24 | 2·0 | 68·3 | 136·6 | 14·0 | 95·3 | 1334·2 | 977 | 1·5 |
| 25 | 2·0 | 95·3 | 190·6 | 14·2 | 25·0 | 375·0 | 196 | — |
| 26 | 1·0 | 25·0 | 25·0 | 2·75 | 57·0 | 156·8 | 627 | — |
| 27 | 2·0 | 57·0 | 114·0 | 9·4 | 38·1 | 358·1 | 314 | 1·5 |
| 28 | 2·0 | 38·1 | 76·2 | 4·3 | 39·3 | 169·0 | 222 | — |
| 29 | 2·0 | 39·3 | 78·6 | 6·0 | 6·1 | 36·6 | 46 | 2·5 |
| 30 | 2·0 | 6·1 | 12·2 | 2·8 | 61·6 | 172·5 | 1414 | 1·6 |
| 31 | 2·0 | 61·6 | 123·2 | 2·7 | 50·1 | 135·3 | 109 | 1·7 |
| 32 | 2·0 | 50·1 | 100·2 | 6·7 | 65·9 | 441·5 | 441 | 2·0 |
| 33 | 2·0 | 65·9 | 131·8 | 2·0 | 122·7 | 245·4 | 186 | 1·7 |

After transfer to the hamsters the cancer cells regularly showed an increase in the relative number of large cells (Fig. 1). This increase in size was extreme during the last passage before the final death of the cancer. After transfer back to mice the resuscitated cancer cells showed normal dimensions.

On transfer of the cancer to the hamster the mitotic index decreased. In most of the series the number of mitosis increased again during the last few passages.

In a previous investigation we found the Ehrlich ascites cancer transferred to hamsters to grow better in females than in males: the yield of cancer cells from the females was as a rule more abundant, and the cancer could be carried through more passages, sometimes through an apparently unlimited number. This also holds for the present investigation. The yield of cancer cells was poorest in Series I–V, which consisted almost entirely of male hamsters, but much better in Series VI, in which all of the animals were female hamsters. In the male series the yield was at most 300 per cent, as against 1000 per cent in the female series.

That the transferred cancer cells grew better in the female hamsters than in the male hamsters was first realised in the course of the present investigation. It was with this in mind that, with the exception of the first two passages, we used only female hamsters in the last series (Series VIII). The cancer cells continued here to grow vigorously during the successive passages (except for a sudden decrease in the eleventh passage) and showed good vitality throughout (Fig. 2). They showed none of the monstruous degenerative forms seen after a few passages in the other series. In addition to the 33 passages recorded in Table I and the diagram the cancer in this series was transferred through a further 11 passages; in these, however, the number of cells was not regularly counted, and the passages are therefore not included in the diagram. In all, Series VIII covered 132 days. It was then interrupted, there being no reason to suppose that prolongation of the experimental period would provide further information.

The fluid of Ehrlich ascites cancer contains a varying number of non-neoplastic cells, mainly leukocytes and mononuclear cells (histiocytes and mesothelial cells). After transmission to hamsters the number was followed through the successive passages until the death of the cancer (Fig. 4). In series I–VII the number of non-neoplastic cells increased rapidly in connection with the regression and death of the cancer cells. In Series VIII the number of non-neoplastic cells increased during the eleventh passage with a correspondingly poor yield of cancer cells. When, during the subsequent passages, the yield became better, the number of non-tumourous cells gradually decreased, and during the twentieth to twenty-sixth passage it lay roughly at the same level as in the donor mouse. During the subsequent passages only a slight increase was noted.

### II. *Did the Cancer Cells Adapt Themselves to the Alien Host?*

(*a*) It is true that the Ehrlich ascites cancer can proliferate in the abdominal cavity of the hamster and cause formation of ascitic fluid, but the growth is of short duration: degenerative changes appear after 4–5 days and the cancer cells soon disappear. The repeated serial transmission had no influence on this course of events: several tests showed that after a total of 250–300 days' sojourn in hamsters the cancer cells were still alien cells for the hamster and caused the formation of antibodies, which after a few days arrested further growth and killed the cancer cells.

(*b*) It is, however, possible that although the cancer cells were still alien for the hamster, they may nevertheless have undergone some " adaptational " change in that they had acquired the power to reproduce themselves more quickly until their growth was arrested by the antibodies. In order to check this possibility, we inoculated 8 mice intraperitoneally with 0·1 ml. of " hamster adapted " ascites cancer (from the twenty-ninth and from the forty-fourth passage, respectively, in Series VIII). The mice were killed 5 days afterwards. Of the ascitic fluid collected, 0·5–1 ml. was injected intraperitoneally into each of 9 male hamsters. The number

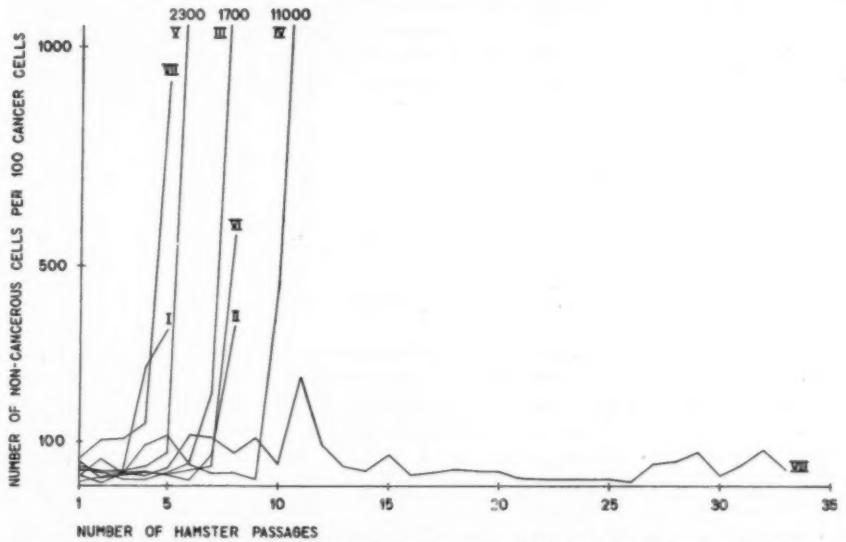


FIG 4.—The number of non-neoplastic cells in the ascitic fluid during the hamster passages. The peak in Series VIII corresponds to the poor yield of cancer cells in the eleventh passage visible in Fig. 3.

of cancer cells inoculated varied between 63·8 and 172·3 $\times$ $10^6$. Three days later the hamsters were killed: the amount of ascitic fluid and its content of cancer cells per ml. was determined. The yield of cancer cells per hundred injected was taken as a measure of growth of the cancer in each animal. The results were compared with those obtained in 17 male hamsters inoculated intraperitoneally with ascitic cancer that had *not* been transmitted through hamsters and which belonged to the same line as that from which the " hamster-adapted " had been derived. The values recorded are given in Table II.

Statistical analysis of variance showed that the difference was clearly significant ($p < 0{\cdot}1$ per cent). The line of ascites cancer that had been transmitted through the successive hamster passages had thus become " adapted ", in that the cancer cells showed increased virulence and reproduced themselves more rapidly in the alien host than did the cancer cells that had not been transmitted through hamsters.

(*c*) In view of the foregoing it was considered worth while determining whether the cancer cells belonging to the " hamster-adapted " line had maintained the

TABLE II.—*The Yield of Cancer Cells in Hamsters Inoculated Intraperitoneally Three days Before with "Hamster-adapted" and "Non-adapted" Mouse Ascites Carcinoma.*

The growth was calculated as percentage of the number of cancer cells inoculated into each hamster.

| "Hamster adapted" ascites cancer growth per cent. | "Non-adapted" ascites cancer growth per cent. |
|---|---|
| 194 | 221 |
| 207 | 431 |
| 365 | 264 |
| 317 | 66 |
| 361 | 148 |
| 315 | 163 |
| 423 | 253 |
| 526 | 100 |
| 183 | 51 |
| | 146 |
| | 24 |
| | 158 |
| | 205 |
| | 173 |
| | 27 |
| | 28 |
| | 98 |
| Mean 321 | Mean 149 |

same power of propagation in *mice* as had the ascites cancer that had been transmitted through mice only, and from which the hamster adapted line had been derived. Ten mice were inoculated intraperitoneally with 0·1 ml. of hamster-adapted ascites cancer ($12{\cdot}6 \times 10^6$ cancer cells) and 10 other mice with 0·1 ml. of non-adapted ascites cancer ($15{\cdot}8 \times 10^6$ cancer cells). The hamster-adapted ascites cancer had been transmitted through 44 hamster passages in Series VIII and then through 31 mouse passages. The mice were killed after 4 days, and the yield of cancer cells was assessed. As before, the increase in the number of cancer cells was calculated as a percentage of the number injected. The results are summarised in Table III.

TABLE III.—*The Yield of Cancer Cells in Mice Inoculated Intraperitoneally 4 Days Before with "Hamster-adapted" and "Non-adapted" Mouse Ascites Carcinoma.*

The growth was caculated as a percentage of the number of cancer cells inoculated into each mouse.

| "Hamster adapted" ascites cancer growth per cent. | "Non-adapted" ascites cancer growth per cent. |
|---|---|
| 574 | 654 |
| 647 | 1696 |
| 923 | 529 |
| 312 | 1135 |
| 1042 | 1690 |
| 411 | 1766 |
| 422 | 892 |
| 972 | 858 |
| 536 | 1325 |
| 876 | 1650 |
| Mean 672 | Mean 1220 |

The non-adapted cancer cells increased to 1220 per cent on the average, while the corresponding figure for the hamster-adapted cells being only 672 per cent. The analysis of variance showed that the difference between the power of reproduction of the cancer cells in the two series was highly probable (0·1 per cent $< p <$ 1 per cent) and thus that after a long sojourn in hamsters ascites cancer appears not to grow so well in mice as it did before.

(*d*) It was pointed out in the foregoing that the Ehrlich ascites cancer can survive an apparently unlimited number of successive transmissions through female hamsters but dies in male hamsters after a few passages. It was therefore considered of interest to investigate whether this difference was also demonstrable in experiments using the hamster-adapted line.

In the twenty-second passage in Series VIII a sub-line of the ascites cancer was started and transferred in a series of male hamsters only. The results are apparent

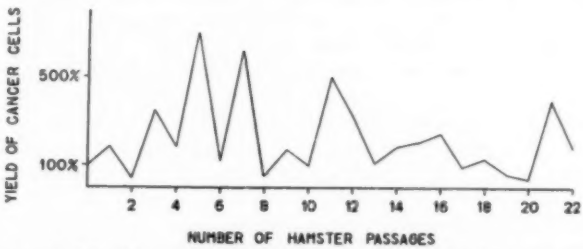


FIG. 5.—The growth of the " hamster-adapted " cancer line (sub-line from the twenty-second passage in Series VIII) in a series of male hamsters. The curve denotes the yield of cancer cells during the successive passages. The yield in each passage was calculated as a percentage of the number of cancer cells inoculated 3 days before.

from Fig. 5 which, like Fig. 3, shows the yield of cancer cells in each passage, calculated as a percentage of the number of cancer cells injected into each hamster. It is evident that the " hamster-adapted " cancer was transmitted without difficulty through 22 male passages. The experiments was interrupted because the cancer still showed undiminished vitality and because it seemed that transplantation could be continued *ad infinitum*. The results will be compared with those obtained in 15 other attempts to transplant Ehrlich ascites cancer in series through male hamsters (12 series published elsewhere (Ahlström and Ising 1955*b*), 3 in the present paper as Series I, IV and V). In these 15 series the cancer could only be passed through at most 11 passages, in the majority of cases only through 3–6 passages.

It appears that the long sojourn of the ascites cancer in hamsters gave it the power to maintain its vitality even under the less favourable conditions implied by the passages through the male hamsters.

## DISCUSSION.

The *solid* form of Ehrlich mouse cancer was transmitted continuously through 300 rat passages for almost 9 years by Putnoky (1930, 1938). He claimed that the mouse tumour had gradually developed rat-protein properties. Also deBalogh (1940) found that some kind of antigenic change had taken place.

Putnoky's interpretation was criticised by Selbie (1936). Recent analyses in association with the hetero-transplantation of human cancers have also revealed the same antigens regardless of the number of generations that the tumour had been grown in the foreign hosts (Korngold and Lipari, 1955). Studies on the effect of tissue culture media on cellular antigens point in the same direction (Landsteiner and Parker, 1940). On the other hand, Langman (1953) was able to demonstrate the presence of cat proteins in transferred rabbit embryo gonads grown in a medium containing cat plasma and cat spleen extract.

In our experiments nothing suggested that the transplanted mouse ascites cancer cells had not maintained their character as alien cells for the hamster. The cancer still behaved like a " regression transplant " with only temporary growth, followed by regression at the same time as when the cancer was tested in the beginning of the experiments.

The accelerated growth in the hamsters after the repeated transfer is consistent with the well known finding that on serial transmission a transplantable tumour often shows increased virulence (Snell, 1953 ; Foulds, 1954). The synchronous reduction in the rate of growth in mice suggests that the change is not a manifestation of increased virulence of the cells by the frequent transfers *per se* but indicates a certain degree of adaptation. That the change in the cancer cells was profound is suggested by the fact that the decreased virulence in mice was evident when the hamster adapted line had been transmitted through 31 mouse passages, by which time it should have been able to readapt itself to its original host.

That the results of transplantation can sometimes vary with the sex of the hosts has been shown by Greene (1940), Foulds (1947) and others. In mice the growth of the Ehrlich ascites cancer does not vary with the sex of the host but it does when the cancer is transferred to the hamster, the heterologous transfer thus revealing latent properties of the tumour. No such difference is demonstrable, however, when the ascites tumour has passed a sufficiently large number of hamster passages.

After transplantation to the hamsters the ascites cancer cells regularly showed an increase in size. In the " hamster adapted " line (Series VIII) the cancer cells did not show the monstrous degenerative forms seen during the last passages in the first 7 series and, judging by the cytologic picture, they could have been transmitted *ad infinitum*.

The fact that the number of non-neoplastic cells did not show any increase in Series VIII also indicates that the cancer cells maintained their vitality. The death of the cancer cells stimulates the accumulation of inflammatory cells in the ascitic fluid (Yoshida, 1949 ; Klein, 1951 ; Ahlström and Ising, 1955).

The change in the tumour cells has been referred to as an adaption, i.e. a modification of the cancer to suit its new environments. Whether it is an expression of a mutation or due to a selection of certain cells better suited than the remainder for multiplication in the foreign host will be discussed in the following article.

## SUMMARY.

1. Ehrlich mouse ascites cancer was transferred from hamster to hamster at 3-day intervals and in this way carried through eight consecutive series of hamster passages ; at the end of the first seven series the cancer was re-transplanted to mice and after a few days transferred to the next series of hamsters. In the eighth series (i.e. the " hamster-adapted " line) the cancer showed good vitality through

44 passages of female hamsters, and it would probably have been possible to continue the transplantation *ad infinitum*. The cancer was carried through in all 92 hamster passages and its total sojourn in the alien host was 276 days.

2. The " hamster-adapted " line showed increased virulence in hamsters and a decreased virulence in mice as compared with the line of origin. It could also be transferred through an apparently unlimited series of male hamsters, while the non-adapted line could only survive a few passages through such animals. The " hamster-adapted " line showed only a relatively slight admixture of non-tumourous cells in the ascitic fluid, in which the cancer cells were well preserved throughout.

## REFERENCES.

AHLSTRÖM, C. G. AND ISING, U.—(1955*a*) *K. fysiogr. Sällsk. Lund. Forh.*, **65**, No. 18.—(1955*b*) *Acta path. microbiol. scand.*, **36**, 415.
DEBALOGH, E.—(1940) *Amer. J. Cancer*, **39**, 45.
FOULDS, L.—(1947) *Brit. J. Cancer*, **1**, 362.—(1954) *Cancer Res.*, **14**, 327.
GREENE, H. S. N.—(1940) *J. exp. Med.*, **71**, 305.
KLEIN, G.—(1951) *Exp. Cell Res.*, **2**, 518.
KORNGOLD, L. AND LIPARI, R.—(1955) *Cancer Res.*, **15**, 159.
LANDSTEINER, K. AND PARKER, R. C.—(1940) *J. exp. Med.*, **71**, 231.
LANGMAN, J.—(1953) *Proc. Kon. Ned. Akad. v. Wetensch.*, **56**, 219.
PUTNOKY, J.—(1930) *Z. Krebsforsch.*, **32**, 520.—(1938) *Amer. J. Cancer*, **32**, 35.
SELBIE, F. R.—(1936) *Ibid.*, **28**, 530.
SNELL, G. D.—(1953) 'The Physiopathology of Cancer.' Chapter 14, p. 338–529. New York (Hoeber).
YOSHIDA, T.—(1949) *Gann*, **40**, 1.

# CHROMOSOME STUDIES IN EHRLICH MOUSE ASCITES CANCER AFTER HETEROLOGOUS TRANSPLANTATION THROUGH HAMSTERS.

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In a previous investigation (Ahlström and Ising, 1955) it was shown that the Ehrlich mouse ascites cancer can survive a long series of short passages through hamsters, and that the cancer undergoes certain biological changes during its sojourn in the foreign host.

The purpose of the present investigation was to reveal and chart any chromosomal change in the tumour stemline during the sojourn of the tumour in the foreign host.

## MATERIAL AND METHODS.

The Ehrlich mouse ascites cancer used was originally obtained from Dr. Klein, Institute for Cell Research, Caroline Institute, Stockholm, since when it has been carried in hybrid mice of the Dobrovolskaja-Zavadskaja strain fed on a standard diet. With a stemline chromosome number of 79–80 (Levan and Hauschka, 1952) this tumour is nearly tetraploid.

Syrian golden hamsters weighing 100–120 g. were used for the heterologous transmission. All of the animals were brought up on a standard diet.

*Chromosome counts.*—Three days after inoculation with the tumour the animal was killed and the ascitic fluid sucked out with a pipette. One droplet of the sample was placed on each of 4–6 slides, were it was immediately mixed with three or four times its volume of acetic orcein (2 per cent orcein in 60 per cent acetic acid). The preparation was allowed to stand for 5–10 minutes, during which it was sometimes heated rapidly for a moment to about 60° C. The cells were then squashed in the ordinary way. Only cells with a smooth, rounded outline of the cytoplasm indicating that no chromosomes had escaped out of the cell during the squashing procedure were accepted for chromosome counting. Exact counts of usually 50–100 plates at metaphase were made for each animal. In some of the samples determinations were made of the frequency of 2 *s* cells (nearly octaploid). The lengths of the chromosomes were measured on camera lucida drawings, each chromosome being placed in the middle of the view field before it was drawn.

*Treatment with colchicine.*—Most of the animals from which specimens were to be collected for chromosome counting received colchicine intraperitoneally in a dose of 0·17 ml. of a 0·006 per cent solution of colchicine per 10 g. bodyweight 18–24 hours before sampling. According to Levan (1954) this dose is above the threshold for c-mitosis in this tumour. Within about 20 hours of its administration colchicine arrests mitosis at metaphase and thereby increases the mitotic index

from the ordinary value of 1 per cent to 30 per cent or more ; it inactivates the central spindle and thereby allows the chromosomes to spread freely in the cell, and it produces a moderate longitudinal contraction of the chromosomes, all factors facilitating the counting. It is known that if the samples are collected 18–22 hours after the administration of colchicine, only one mitotic cycle is influenced, but that prolongation of the interval until the commencement of a second mitotic cycle results in the appearance of many deviating chromosome numbers making such sample useless for chromosome studies.

No colchicine-treated tumours were used for propagation. The chromosome studies were made on duplicate animals used exclusively for this purpose.

## RESULTS.

### A. *Changes in chromosome number.*

1. *The original tumour.*—During the years the Ehrlich ascites mouse tumour has been carried in mouse passages at the institute of Pathology, Lund, chromosome analyses (by Ahlström) have always shown it to be nearly tetraploid. During the autumn of 1954 the chromosomes were counted in 50–100 metaphase plates from each of eight animals. The appearance of the chromosomes in a cell from a specimen collected at that time from one of the colchicine-treated animals is reproduced in Fig. 1*a*.

The results of these counts are given in the top of Table I, which shows the usual variation about a mode of 80 in six of the animals and of 79 in two, with an overall average chromosome number of 79·6 (range : 79·0 to 80·1). The distribution of the chromosome numbers in the total material is given in Fig. 4.

2. *The tumour in hamster passage.*—After seven months' repeated serial transfer through hamsters, interrupted only occasionally by a few days' mouse transfer, the first chromosome counts were made ; later, during the autumn of 1954, four additional counts were made at intervals of two to three weeks. The results are summarised in the middle part of Table I, from which it is clear that this repeated serial transfer of the tumour through hamsters was accompanied by a change in the distribution of the chromosome numbers : the mode for all five having decreased to 76 and the average chromosome number now showing only a narrow variation round 76·2. This decrease in stemline number from 79–80 to 76 is clear from Fig. 4, which also suggests a narrowing of the scatter of the chromosome numbers, the modal class containing as many as 39 per cent of all cells, as against only 25 per cent in the mouse series. A microphotograph of a 76-chromosome cell at metaphase, taken from a specimen collected during passage of the tumour through hamsters is reproduced in Fig. 1*b*.

3. *The tumour after retransplantation to mice.*—After a total of 245 days' passage through hamsters the tumour was re-inoculated into mice of the original strain. Chromosome counts were made at certain intervals (Table I and bottom of Fig. 4). The mode found for all of the five samples studied was 76, the averages varying from 75·8 to 76·5. The changes that the stemline had undergone during its passage through hamsters had thus evidently persisted through 5 months' subsequent passage through mice. The scatter of the chromosome numbers had become still narrower, 51 per cent of the cells now having the *s* number. The appearance of a tumour cell with 75 chromosomes is seen in Fig. 1*c*.

TABLE I.—*Chromosome Number Distributions.*

| Chromosome number per cell. | | <71 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | >85 | Total. | Average Chromosome number per cell. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **In mouse.** | | | | | | | | | | | | | | | | | | | | |
| Number of cells | Mouse 1 | — | — | 1 | — | — | 3 | 1 | 3 | 16 | 19 | 23 | 11 | 13 | 5 | — | — | 4 | 99 | 80·07 |
| | „ 2 | 1 | — | 2 | 1 | — | 1 | 4 | 7 | 18 | 11 | 18 | 13 | 11 | 8 | 4 | — | 1 | 100 | 79·57 |
| | „ 3 | 2 | — | — | — | 1 | — | — | 3 | 6 | 13 | 9 | 6 | 6 | 1 | — | 2 | 1 | 50 | 78·96 |
| | „ 4 | — | — | — | 1 | — | 3 | 2 | 10 | 11 | 19 | 26 | 16 | 9 | 2 | — | — | — | 99 | 79·38 |
| | „ 5 | — | 1 | — | 1 | 1 | — | 4 | 5 | 12 | 19 | 31 | 9 | 7 | 3 | 5 | — | 2 | 100 | 79·68 |
| | „ 6 | — | — | — | — | 2 | — | 1 | 3 | 14 | 18 | 32 | 16 | 10 | 3 | — | 1 | — | 100 | 79·79 |
| | „ 7 | — | — | — | — | 1 | 3 | — | 5 | 4 | 8 | 16 | 6 | 2 | 2 | — | 2 | 1 | 50 | 79·74 |
| | „ 8 | — | — | — | — | 1 | 1 | 5 | 9 | 12 | 27 | 22 | 10 | 9 | 1 | 2 | — | 1 | 100 | 79·45 |
| Absolute | | 3 | 1 | 3 | 3 | 6 | 11 | 17 | 45 | 93 | 134 | 177 | 87 | 67 | 25 | 11 | 5 | 10 | 698 | 79·58 |
| Per Cent | | 0·4 | 0·1 | 0·4 | 0·4 | 0·9 | 1·6 | 2·4 | 6·5 | 13·3 | 19·2 | 25·4 | 12·5 | 9·6 | 3·6 | 1·6 | 0·7 | 1·4. | 100 | — |
| **In hamster.** | | | | | | | | | | | | | | | | | | | | |
| Number of cells | After— 234 days | 3 | — | 2 | — | 9 | 12 | 35 | 16 | 9 | 9 | 2 | 1 | 1 | — | — | — | — | 99 | 76·04 |
| | 240 „ | 1 | 1 | — | 1 | 4 | 7 | 15 | 8 | 4 | 1 | 4 | — | — | 1 | — | — | — | 47 | 76·26 |
| | 252 „ | 1 | — | — | 2 | 5 | 18 | 39 | 18 | 9 | 5 | 1 | 1 | — | 1 | — | — | — | 100 | 76·24 |
| | 277 „ | — | — | — | — | 1 | 6 | 33 | 7 | 2 | — | 1 | — | — | — | — | — | — | 50 | 76·14 |
| | 331 „ | — | — | — | 3 | 10 | 11 | 33 | 22 | 15 | 2 | 1 | 2 | 1 | — | — | — | — | 100 | 76·38 |
| Absolute | | 5 | 1 | 2 | 6 | 29 | 54 | 155 | 71 | 39 | 17 | 9 | 4 | 2 | 2 | — | — | — | 396 | 76·21 |
| Per Cent | | 1·3 | 0·3 | 0·5 | 1·5 | 7·3 | 13·6 | 39·1 | 17·9 | 9·8 | 4·3 | 2·3 | 1·0 | 0·5 | 0·5 | — | — | — | 99·9 | — |
| **Back in mouse.** | | | | | | | | | | | | | | | | | | | | |
| Number of cells | After— 30 days | 1 | — | — | 2 | 2 | 7 | 21 | 8 | 4 | 1 | 1 | — | — | 1 | 1 | 1 | — | 50 | 76·48 |
| | 46 „ | 1 | — | — | — | 1 | 7 | 11 | 1 | 3 | — | 1 | — | — | — | — | — | — | 25 | 75·80 |
| | 66 „ | — | — | — | — | 1 | 8 | 32 | 7 | 1 | 1 | — | — | — | — | — | — | — | 50 | 76·04 |
| | 93 „ | — | — | 1 | — | 6 | 26 | 52 | 6 | 6 | 1 | 1 | 1 | — | — | — | — | — | 100 | 75·88 |
| | 146 „ | — | — | 2 | — | 6 | 13 | 49 | 17 | 11 | 2 | — | — | — | — | — | — | — | 100 | 76·12 |
| Absolute | | 2 | — | 3 | 2 | 16 | 61 | 165 | 39 | 25 | 5 | 3 | 1 | — | 1 | 1 | 1 | — | 325 | 76·06 |
| Per Cent | | 0·6 | — | 0·9 | 0·6 | 4·9 | 18·8 | 50·8 | 12·0 | 7·7 | 1·5 | 0·9 | 0·3 | — | 0·3 | 0·3 | 0·3 | — | 99·9 | — |

A small proportion of cells with a chromosome number of 2 *s* (nearly octaploid) was regularly seen throughout all three series. In the original tumour this proportion was estimated at less than 1 per cent and appeared to be slightly larger (1–5 per cent) in the 76 chromosome line, but the difference was not statistically significant. The appearance of a nearly octaploid cell is reproduced in Fig. 2*a*.

B. *Chromosomal morphology.*

The tumour was studied for any changes in chromosomal morphology accompanying the loss of the four chromosomes during the passage of the tumour through the hamsters. Complete idiograms from ten random samples of the original tumour and from nine samples collected during the passage of the tumour through hamsters were analysed and measured. One idiogram from each occasion is exemplified in Fig. 3. As expected, no consistent differences were found between the two groups of idiograms. All of the chromosomes were telocentric, the variation in size, from the smallest to the largest chromosome, in each idiogram was continuous. No individual chromosomes could be identified.

No statistical difference was found between the two groups with regard to distribution of the chromosomes according to length. The average variation in length of the chromosomes within each plate was the same in both groups (1·5 $\mu$–5·6 $\mu$). The total chromosome length per cell was 233·8 $\mu$ in the mouse group and 262·7 $\mu$ in the hamster group, the average length of the individual chromosome thus being 2·95 $\mu$ and 3·44 $\mu$, respectively. This difference was, however, not statistically significant, and considering the unavoidable slight variation in the performance of the squashing, the possible variation in the response of the host to colchicine and differences in the degree of contraction of the chromosomes, and other unknown or uncontrollable factors, the true difference, if any, was probably much less than that suggested by the figures found. At this stage, however, it might be convenient to mention that the cells in samples collected during passage of the tumour through hamsters were slightly larger than in samples taken from mice (Ahlström and Ising, 1955).

The contraction of the chromosomes in the colchicinised animals was only moderate, the values found for a cell from an untreated animal of each group lying well within the variation of those found for the cells from the colchicinised animals. The measurements recorded in the present investigation agree, according to Levan (1954), well with those found for the same tumour grown in Philadelphia.

In the hamster series chromosomes of strikingly abnormal appearance were not uncommon. An example of a cell with two giant chromosomes twice to four times the ordinary average chromosomes length is given in Fig. 2*b*. In one sample from the hamsters they were seen in about 7 per cent of the chromosomes at metaphase. Such giant chromosomes were also occasionally seen in the original mouse series.

The interesting chromosomal abnormality depicted in Fig. 2*c* was found in a cell of the hamster series. In this 80-chromosome metaphase four of the chromosomes had contracted less than the remainder : they were thinner and longer, and consisted of alternating lumps of chromatic matter and almost unstained material. Similar instances of negative heteropyknosis in single chromosomes within otherwise normal plates have been illustrated by Tjio and Levan (1954) within strains of the hyperdiploid Ehrlich cancer. Such differences in the degree of contraction in one and the same cell might be interpreted as a sign of the chromosomes in the tumour cells being in a less stable state of equilibrium.

## DISCUSSION.

The change observed in the chromosome number from 79–80 to 76 in the stemline of the Ehrlich mouse ascites tumour on repeated serial transmission through hamsters is highly suggestive. When carried continuously in mice of our strain the original tumour maintains its stemline number of 79–80. It should be mentioned that heterologous transplantation of the same Ehrlich strain to hamster has been repeated twice at our laboratory: in one of the series the stemline chromosome number decreased from 80 to 76–77 in 102 days or 34 hamster passages, and in the other series from 80 to 76–77 in 66 days or 22 passages. Details of these two series will be published later. This decrease in a tumour that has maintained its original stemline chromosome number during years of serial transfer in mice must surely be ascribed to environmental changes encountered in the foreign host.

Recent chromosomal investigations of ascites tumours in rats and mice strengthen the concept of the cancer cell population as a genetically highly heterogeneous mosaic (Hauschka, 1952). The surprising measure of constancy still characteristic of transplantable tumours is due to the equilibrium existing between factors promoting and counteracting genetic variability. The factor mainly responsible for the constancy of the properties of the tumour is a superior viability of the stemline genotype. As long as the environment is one and the same, the stemline of such an old, well established tumour as the nearly tetraploid Ehrlich mouse ascites tumour, is probably superior to any deviating genotype that might appear. In the present investigation the transition to the new stemline was fortunately microscopically demonstrable by the decrease in chromosome number. However, nothing is known about the sequence of events or the mechanisms involved in this transition.

Radical environmental changes, such as those involved by transfer to a foreign species, surely will upset the above mentioned equilibrium. New genotypes may be able to compete favourably with those of the old stemline: a selection is started in the cell population.

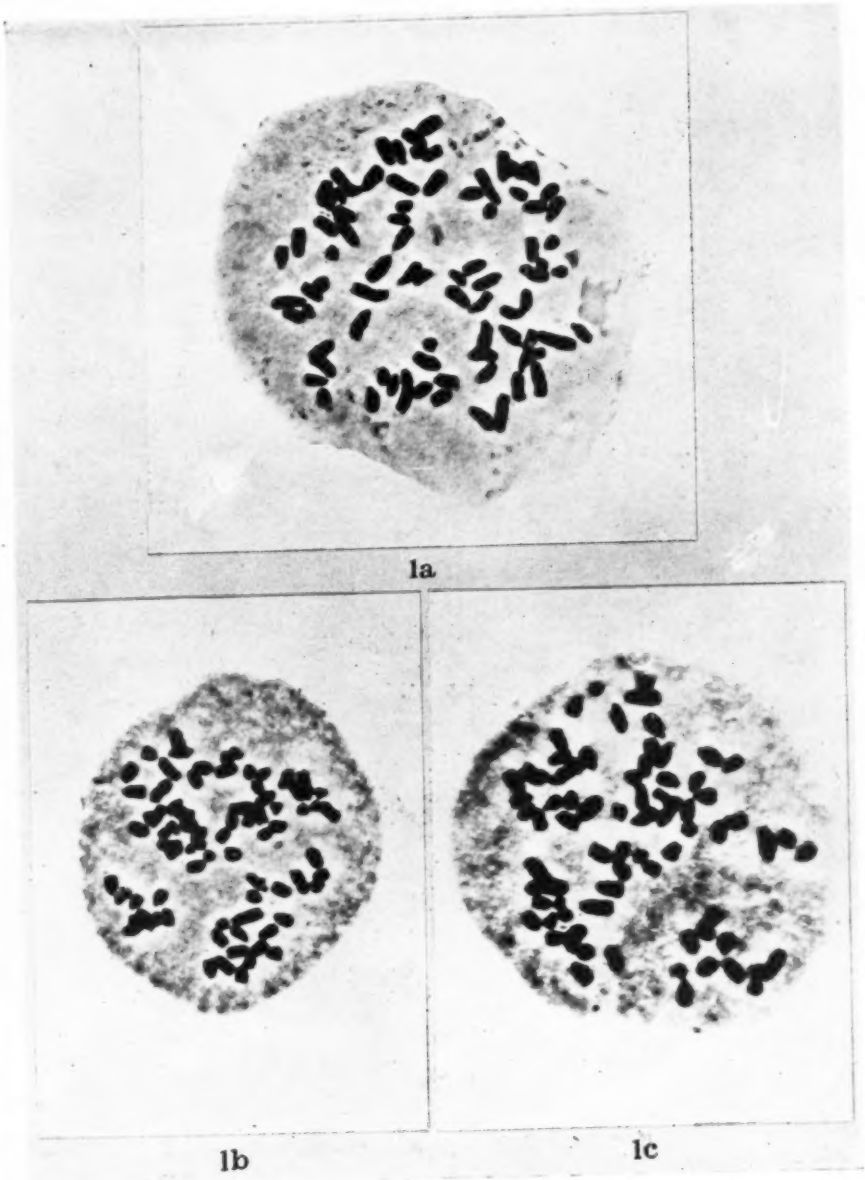
Simultaneous mutations enhancing the viability of the new stemline in being may accumulate. A general increase in the rate of mutation is probably due to the change in environment imposed on the population by transfer of the tumour to a foreign host.

However, we do not know whether the disappearance of the four chromosomes from the original stemline implies a true loss of so much genetic material, or whether the loss is accompanied by translocations of the genic material in the chromosome

## EXPLANATION OF PLATES.

FIG. 1.—(*a*) Metaphase of Ehrlichs nearly tetraploid ascites cancer in mouse. Aceto-orcein staining. ×880. (*b*) Metaphase of the same cancer after serial transplantation through hamsters. Aceto-orcein staining. × 880. (*c*) Metaphase of " the adapted " cancer after retransplantation to, and serial transplantation through mice. Aceto-orcein staining. × 880.

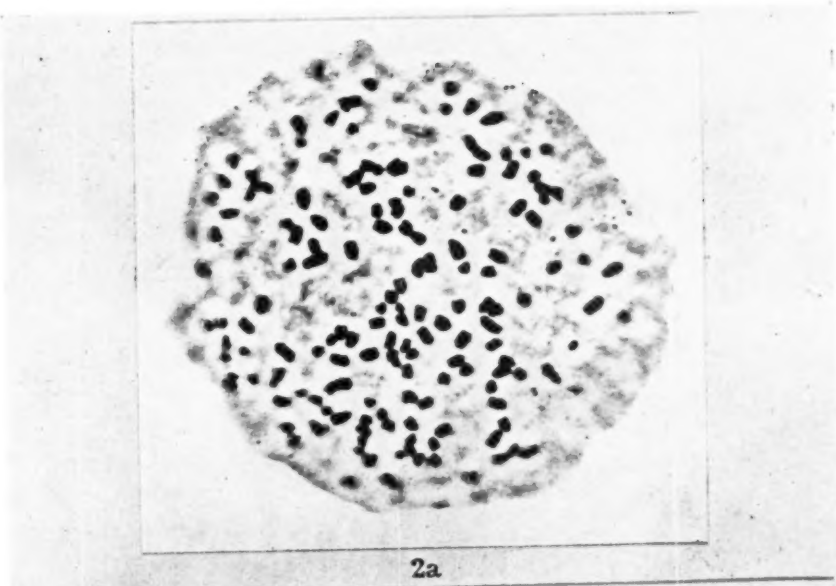
FIG. 2.—(*a*) Metaphase of 2 *s* (octaploid) cell from Ehrlich nearly tetraploid ascites cancer after serial transplantation through hamsters. Aceto-orcein staining. × 880. (*b*) Metaphase with 2 large chromosomes in the same cancer after serial transplantation through hamsters. Aceto-orcein staining. × 880. (*c*) Metaphase cell of the same cancer after hamster passages: negative heteropyknosis. Aceto-orcein staining. × 880.



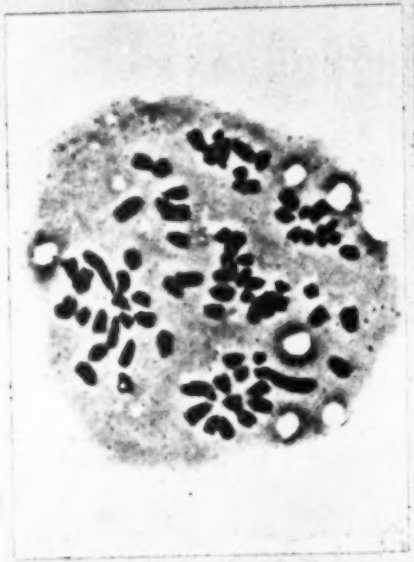
1a

1b

1c



2a



2b

2c

s
d
s

a

set so that the actual loss would comprise little more than four centromeres. The decrease in the quantity of chromatic substance due to the loss of the four chromosomes is too small to be demonstrable by DNA analysis.

It was interesting to note that the stemline number of 76 still persisted 3 months after the tumour had been transferred back to mice. This suggests that the chromo-

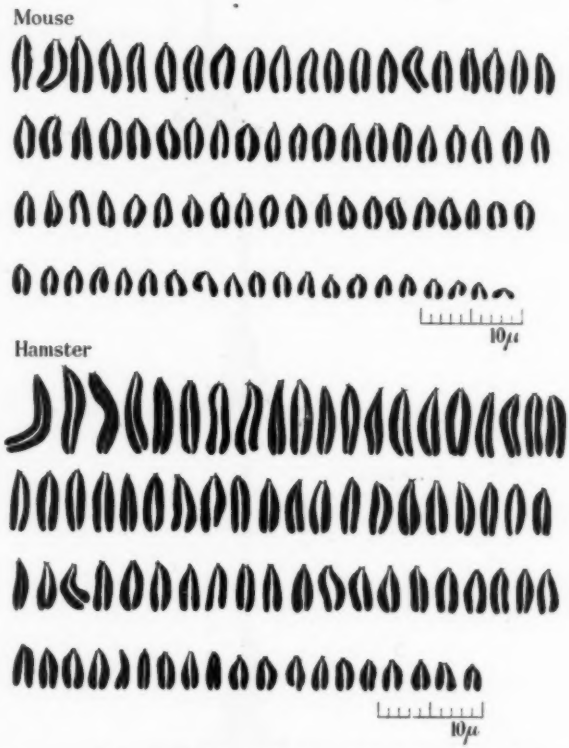



FIG. 3.—(*a*) Idiogram analysis of Ehrlich nearly tetraploid ascites cancer in mouse. Colchicine treated. Camera lucida drawing. (*b*) Idiogram analysis of Ehrlich ascites cancer after 240 days' hamster passage. Colchicine treated. Camera lucida drawing.

some number of this nearly tetraploid tumour is more liable to decrease than to increase.

In the first part of this paper it was reported that the tumour gained in virulence in the hamster. But we do not know with certainty whether this phenomenon is related to the chromosomal change. It should be recollected, however, that the occurrence of increasing virulence in most young tumours is regularly accompanied by a decrease in host specificity (Hauschka and Levan, 1953 ; Klein 1955), and by changes in chromosome number (Hauschka and Levan, 1953). It therefore

appears reasonable to assume that the increased virulence of the tumour in hamsters was acquired in association with the development of the new stemline genotype, a development reflected by the change in the stemline chromosome number.

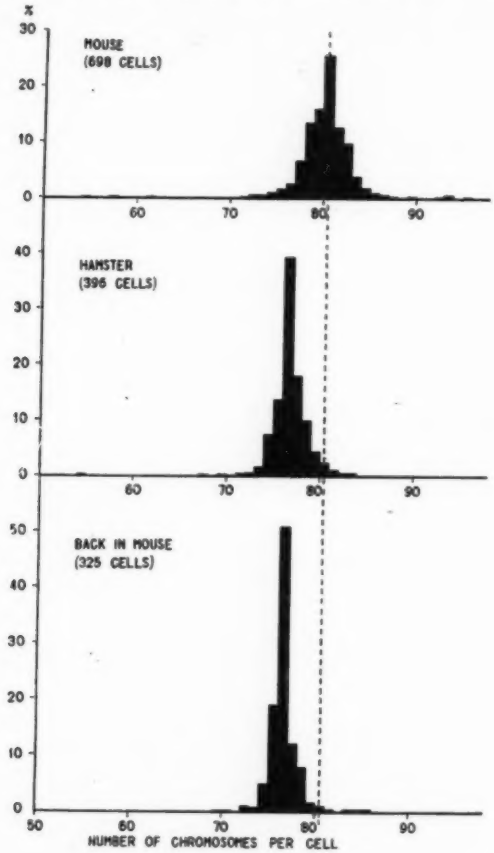



FIG. 4.

SUMMARY.

After having been carried for seven months in serial passages in hamster, the near-tetraploid Ehrlich mouse ascites tumour originally having a stemline chromosome number of 79–80 was shown to have shifted over to a stemline number of 76 chromosomes. After back-transfer for 30 passages in mouse hosts of the original strain the lower stemline number was still maintained. Two independent repetitions of this experiment have given similar results. No morphological difference in the chromosomes between the 76 and the 80-chromosome tumour type could be demonstrated.

## REFERENCES.

AHLSTRÖM, C. G. AND ISING, U.—(1955) *K. fysiogr. Sällsk. Lund Förh.*, **65**, No. 18.—(1955) *Acta path. microbiol. scand.*, **36**, 415.
HAUSCHKA, T.—(1952) *Cancer Res.*, **12**, 615.
*Idem* AND LEVAN, A.—(1953) *Exp. Cell Res.*, **4**, 457.
KLEIN, E.—(1955) *Ibid.*, **8**, 213.
LEVAN, A.—(1954) *Hereditas*, **40**, 1.
*Idem* AND HAUSCHKA, T.—(1952) *Ibid.*, **38**, 251.
TJIO, J. H. AND LEVAN, A.—(1954) *K. fysiogr. Sällsk. Lund Förh.*, **65**, No. 15.

# RADIOBIOLOGY OF THE C3H MOUSE MAMMARY CARCINOMA: SPONTANEOUS MUTATION IN A HOMOPLAST RESULTING IN INCREASED RADIOSENSITIVITY.

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AN essential requisite of experimental oncology, if it is to have any applicability to human cancer, is a host-tumour relationship approaching as closely as possible that existing in autogenous or spontaneous tumours. While transplantation of isogenic tumours within an inbred strain of animals has been assumed to fulfil this requirement, the immunogenetic identity of serially transmitted tumours may diverge progressively from that of the host, eventually giving a "foreign" or immunizing tumour, too readily cured by facile procedures. The occurrence of spontaneous mutations in serially transmitted tumours is, in fact, one of the major pitfalls encountered in experimental cancer research (Hauschka, 1952; Snell *et al.*, 1946; Strong, 1949). It seems probable that many confusing reports of resistance to isogenic tumours arising in inbred strains can be ascribed to this phenomenon.

We have attempted to eliminate this difficulty by restricting transplantation to no more than one or two subpassages from the host of origin. In so doing one requires a constant source of more or less identical spontaneous tumours, and the C3H adenocarcinoma seemed entirely adequate in this respect. We have found that autogenous mammary tumours arising in old females of the factor-bearing C3H/Cg strain have exhibited a constant morphology and radiosensitivity. These tumours have provided the source material for first and second passage homoplasts, the latter constituting the pool of experimental tumours used in this laboratory. In spite of these precautions, however, a spontaneous mutation of a mammary tumour occurred at a first passage, the only instance thus far detected in our series of 140 autogenous tumours transplanted into about 1500 recipient mice over a period of five years. As will be shown, the mutant, though morphologically identical with the original tumour, exhibited a marked change in radiosensitivity. It seemed that unless such an occurrence were recognised at the outset, it might invalidate experiments on the curability of any particular transmitted tumour. The characteristics of this variant were therefore investigated further and are described below. The term "mutation" is here used to characterize this hereditary cellular change, without necessarily implying a specific gene mutation.

## MATERIALS AND METHODS.

*Animals.*—As in the previous series of experiments, young mice of both sexes of our factor-bearing substrain C3H/Cg were used.

*Tumour.*—The mutation arose in a homoplast growing in a mouse which had been inoculated in a routine first-passage transmission with a fragment of an

autogenous mammary carcinoma from a female of this strain. Tumours borne by co-recipients inoculated at the same time from the same source showed no obvious changes. The mutant-tumour-bearing mouse was then inadvertently used as a donor for a routine second passage, in which *all* the resultant tumours exhibited the change. That a mutation had in fact arisen was indicated by the obviously decreased latent period, increased growth rate, and markedly altered radiosensitivity of the tumour when treated *in situ*. Following these observations, the tumour was transplanted through 8 successive passages, consistently displaying the foregoing characteristics.

Of a total of 80 mice inoculated, the mutant tumour " took " in only 70 cases, indicating an initial resistance rate of 12½ per cent. This differs remarkably from our standardised C3H tumour, in which failure of an inoculum to grow is an extreme rarity, probably less than once in 1000 implants. By repeated challenge of the initially resistant mice further successful " takes " were obtained in about one-half of these animals.

On histological examination, the mutant tumour showed a general structure indistinguishable from that of our previously standardised C3H adenocarcinoma, except for a greater number of mitotic figures commensurate with its higher growth rate.

*Radiation factors.*—As in all previous experiments, roentgen rays generated at 240 kVp., with no added filters, HVL 0·34 mm. Cu, FSD 25 cm., were used. The dose rate was 500r/min. for treatment *in situ* with the 2 cm. diameter applicator (Cohen and Cohen, 1953).

*Experimental design.*—A total of 57 tumours of the mutant line were irradiated *in situ* at 3-dose levels corresponding to those used in previous series—4200r, 3500r, and 3000r. The proportion of cures at each dose was tabulated (Table I) and plotted on the log-probit graph (Fig. 1) for comparison with data previously obtained for our standardised C3H tumour. On the analogy of a 6-point assay, the magnitude and significance of the change in radiosensitivity was estimated by standard statistical methods.

## RESULTS.

### *Tumour size and growth rate.*

The rate of growth of this mutant tumour line was much more rapid than that of the previously standardised tumour. The latent interval between the time of implantation and the appearance of a palpable " take " was generally under 7 days, compared with a period of 7 to 15 days for the standard tumour. Similarly, the interval between implantation and irradiation in this experiment ranged from 10 to 25 days, while the usual C3H tumour is rarely large enough for treatment before the 30th day after implantation. Despite this short interval, the average size of these tumours at the time of treatment was somewhat larger than that in previously reported series. All tumours were roughly spheroidal in shape, and their volumes were estimated by measurement at the time of treatment from the formula $V = \frac{\pi}{6} ab^2$, where $a$ is the axial length and $b$ the equatorial diameter of the spheroid. The average tumour volume at treatment was about 1000 mm.$^3$, though at no time was a tumour greater than 2 cm. in its largest diameter, or more than 4000 mm.$^3$ in volume, used. Since larger tumours may be expected on theoretical grounds to be less easily eradicated, the unavoidable discrepancy in

size between the mutant and control groups actually enhances the significance of the observations.

In order to compare growth rates we assumed an exponential increase ($V.e^{gt}$) of tumour volume ($V$) with time in days ($t$), so that the growth constant ($g$) could be estimated for each mouse from measurements of tumour size at treatment. While the average value for the growth constant of our standard C3H tumour growing in this strain of C3H mouse is $g = 0{\cdot}20$ ($\pm 0{\cdot}014$) day$^{-1}$ (Cohen and Cohen, 1954*a*), the tumour doubling its volume approximately every 4 days, the growth of the mutant line corresponds to a value of $g = 0{\cdot}50$ ($\pm$ 0·04) day$^{-1}$, this tumour doubling its volume every $1\frac{1}{2}$ days. The mutant tumour thus grows about $2\frac{1}{2}$ times as rapidly as our standardised mammary carcinoma, a highly significant ratio despite our relatively crude methods of measurement.

*Radiosensitivity.*

The results of treatment *in situ* of the mutant line adenocarcinoma at three dose levels are shown in Table I. It will be noted that a high proportion of cures is obtained at a dose of only 4200r, and a few even at 3500r, although both these

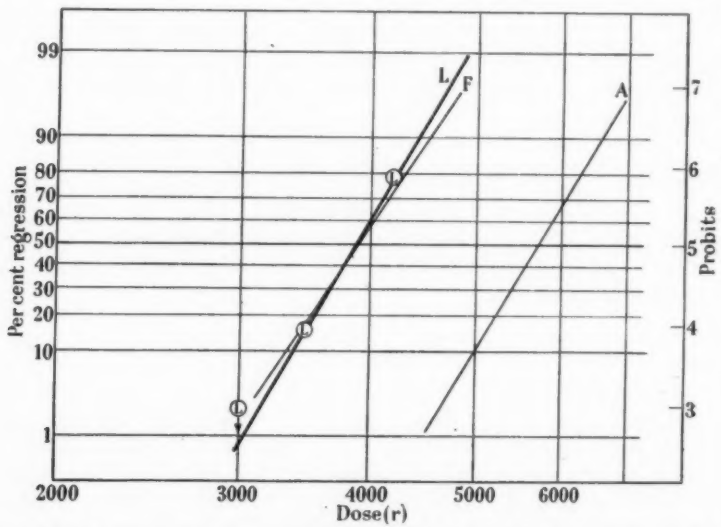


FIG. 1.—Probit diagram showing response of the mutant line adenocarcinoma (L) to irradiation *in situ* compared with the standardised isogenic tumour in C3H mice (A) and in factor-free $F_1$ hybrid hosts (F).

doses are known to be completely ineffective when the usual standardised tumour is treated in the C3H host. For the purpose of comparison, the data are shown in the probit diagram (Fig. 1) together with the previously established regression lines for our standard C3H control series and a factor-free $F_1$ hybrid host. Analysis of the data shows that the median effective dose for the mutant tumour treated *in situ* is 3900r ($\pm$ 40*), compared to 5700r for the control series. The ratio of these factors, a measure of the difference in radiosensitivity, is 1·46

($\pm$ 0·05*), indicating a highly significant increase ($p < 0·0001$) in radiosensitivity of the mutant line. The coefficient of variation, as evidenced by the slope of the line (line L, Fig. 1), is less than 10 per cent, indicating as consistent a response as that obtained with the control series (line A, Fig. 1).

TABLE I.—*Treatment* In Situ *of the Mutant Line Adenocarcinoma in C3H Mice.*

| Dose (r). | Number or mice. | Number cured. | Cures (per cent) | Cure rates expected with non-mutant tumour. |
|---|---|---|---|---|
| 4200 | 34 | 27 | 79 | <1 % |
| 3500 | 13 | 2 | 15 | <0·01 % |
| 3000 | 10 | 0 | 0 | — |

The response of the mutant tumour bears comparison with that obtained when the C3H carcinoma is grown in factor-free (CBA × C3H)$F_1$ hybrid hosts (Cohen and Cohen, 1954*a*), as shown in Fig. 1 (line F). The median lethal doses and their coefficients of variation are seen to be practically identical in the two cases.

Subsequent challenge with a second inoculum of the same mutant line in mice which had been cured by irradiation resulted in active growth in all cases, indicating that no absolute immunity to the tumour had been induced.

## DISCUSSION.

The frequency of tumour mutation is such that continual variation seems to be more the rule than the exception. A wide range of recognisable hereditable changes in the character of transmitted tumours has been recorded and ascribed to genic mutation, random variation, or selective adaptation. The greatest potentiality for variation appears to exist where tumours are being transmitted through heterozygous hosts partially or wholly alien to the strain of origin (Barrett and Deringer, 1950, 1952; Barrett, Deringer and Hansen, 1953). However, even isogenic tumour lines can differentiate either as a result of prolonged serial transmission (Foley, 1952), or from the mutagenic action of the carcinogenic hydrocarbon used to induce the primary growth, after only a few passages in the inbred strain of origin (Foley, 1953).

Compared to the parent tumour, a mutant line may show one or more of the following aberrations: a change in the proportion of successful takes on homoiotransplantation; an increased rate of growth of the established tumour; an increased tendency to metastasise; a loss of host specificity with the ability to grow in relatively alien strains (Strong, 1949); a tendency to regress after trauma and occasionally to induce absolute immunity to further implants of the same tumour; transformation from one morphologic type to another (see Dunham and Stewart's survey, 1953); acquisition of the facility to produce ascites (Klein and Klein, 1951; Klein, 1955); shift in the modal value of chromosome number to the tetraploid state (Hauschka and Levan, 1953); and, as we have shown, a marked change in radiosensitivity.

The findings reported here illustrate a change that arose in an isogenic tumour even when the host strain of origin was rigidly inbred and prolonged serial

* Standard error of median and of ratio of medians.

transmission was avoided. The proportion of takes in the strain of origin was significantly reduced, the curability was greatly enhanced with a general absence of metastases, and a few immune mice observed, all indicating an increased resistance on the part of the host against this tumour. It is interesting to note that the characteristics of the mutant were, in a sense, contradictory: the decreased host susceptibility being associated with an increased growth rate. This situation is reminiscent of the well-known clinical observation that the more rapidly growing anaplastic tumours generally have a greater radiosensitivity.

Since none of the mice inoculated in the first passage showed any overt change in growth rate or radiosensitivity, the original autogenous tumour used as the source was probably no different from all others in this series of experiments. The fact that all the tumours in the second passage were obviously atypical, indicates that the mutation must have appeared suddenly after implantation of the tumour into the particular mouse used as the donor for the second passage. The change was obviously hereditary, persisting through eight transplant generations.

As previously described (Cohen and Cohen, 1954), even a minor antigenic difference between host and tumour results in a perceptible increase in radiosensitivity. The present experiment demonstrates an increased radiosensitivity as a result of spontaneous change in the character of the tumour transplant. The fact that the radiosensitivity of the mutant tumour closely resembles that of the originally standardised tumour grown in the factor-free $F_1$ hybrid hosts, suggests that the same degree of antigenic difference exists between the mutant tumour and the homozygous host as between the isogenic tumour and a heterozygous host. It appears that the radiosensitivity of a tumour is a delicate index of host-tumour diversification, irrespective of whether the initial change occurs in host or tumour. As previouly suggested (Cohen and Cohen, 1954*c*), the same immunological mechanism operates in both instances.

SUMMARY.

The characteristics of a sudden spontaneous mutation, occurring in one first passage C3H mouse mammary carcinoma homoplast, are described. On further transmission, 12½ per cent of the inbred C3H hosts were found to be initially resistant to this tumour. The mutant tumour was morphologically similar to other mammary tumours of the strain, but its growth rate was 2½ times that of non-mutant tumours. When treated *in situ*, the $LD_{50}$ proved to be 3900r as compared to 5700r of non-mutant tumours. The change appeared to be permanent, the tumour displaying consistent behaviour through eight transplant generations.

We are indebted to Dr. J. F. Murray for providing facilities at the South African Institute for Medical Research where the C3H/Cg mouse colony is maintained, and to Mrs. Shelley Jacobson for her invaluable skilled assistance in the upkeep and breeding of this colony.

## REFERENCES.

BARRETT, M. K. AND DERINGER, M. K.—(1950) *J. nat. Cancer Inst.*, **11**, 51.—(1952) *Ibid.*, **12**, 1011.

*Iidem* AND HANSEN, W. H.—(1953) *Ibid.*, **14**, 381.

COHEN, A. AND COHEN, L.—(1953) *Brit. J. Cancer*, **7**, 231.—(1954*a*) *Ibid.*, **8**, 303.—(1954*b*) *Ibid.*, **8**, 313.—(1954*c*) *Ibid.*, **8**, 522.
DUNHAM, L. J. AND STEWART, H. L.—(1953) *J. nat. Cancer Inst.*, **13**, 1299.
FOLEY, E. J.—(1952) *Proc. Soc. exp. Biol., N.Y.*, **79**, 151.—(1953) *Cancer Res.*, **13**, 835.
HAUSCHKA, T. S.—(1952) *Cancer Res.*, **12**, 615.
*Idem* AND LEVAN, A.—(1953) *Exp. Cell Res.*, **4**, 457.
KLEIN, E.—(1955) *Exp. Cell Res.*, **8**, 188, 213.—(1955) 'Transformation of Solid into Ascites Tumors.' Uppsala (Almqvist & Wiksell).
KLEIN, G. AND KLEIN, E.—(1951) *Cancer Res.*, **11**, 466.
SNELL, G. D., CLOUDMAN, A. M., FAILOR, E. AND DOUGLAS, P.—(1946) *J. nat. Cancer Inst.*, **6**, 303.
STRONG, L. C.—(1949) *Brit. J. Cancer*, **3**, 97.

# CARCINOGENIC AGENTS AND THE METABOLISM OF ASCORBIC ACID IN THE GUINEA-PIG.

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THE action of some carcinogenic agents upon the guinea-pig was examined in view of (1) the resistance of this species to some such agents and (2) the resemblance of the guinea-pig to man in its inability to synthesise ascorbic acid.

Earlier investigations upon the ascorbic acid content of the liver gave the following results :

(1) An increase in pregnancy in mice and in rats (Kennaway and Kennaway, 1944 ; Kennaway and Tipler, 1947).

(2) In mice, an increase following the injection (subcutaneous or intraperitoneal) of seven carcinogenic polycyclic hydrocarbons, of 1 : 2-benzanthracene and of stilboestrol, while 9 : 10-dimethylanthracene (carcinogenic to skin), anthracene, phenanthrene, and naphthalene had no such effect (Kennaway, Kennaway and Warren, 1944).

(3) 2-Acetamidofluorene when injected (subcutaneously and intraperitoneally) caused an increase in male, but not in female, CBA mice, and in black-and-white, and in Wistar rats, and an increase also when fed to Wistar rats. The 7-OH derivative had no such effect except for a very slight increase in black-and-white rats (Daff, Hoch-Ligeti, Kennaway and Tipler, 1948).

(4) The fall in ascorbic acid content of the liver in mice on a vitamin-free diet is prevented by the addition of yeast, milk or methionine ; riboflavin, below a certain dosage, is equally effectual (Kennaway and Daff, 1946).

The action of some of these factors has now been tested in guinea-pigs, in which species the inability to synthesise ascorbic acid makes the metabolism of this compound similar to that in man. All amounts of ascorbic acid in tissues are stated below as $\mu$g./g. wet weight. The effects of some factors upon the concentration of ascorbic acid in the liver in guinea-pigs, and in mice, are compared in Table I.

*Technique.*

About 1g. liver was homogenised in 3 per cent metaphosphoric acid, centrifuged, and re-extracted twice ; the extract was titrated with 2 : 6-dichlorophenolindophenol.

*Pregnancy.*

The concentration of ascorbic acid in the liver in seven pregnant guinea-pigs ranged from 81 to 150, mean 117, and three found to be pregnant when receiving arachis oil gave 158, 126, 134 ; mean 139. For controls, we have only five receiving arsenic (see below), range 84–470, and three exposed once to X-radiation, range 119 to 356, the mean for the eight animals being 214. This very wide range does

not allow any final conclusions, but there is no indication of the increase in pregnancy shown by mice and by rats (Table I ; for references see above). The seven male controls show a similar range (99 to 352, mean 202).

*Intraperitoneal injections of hydrocarbons in arachis oil.*

(1) 1.2.5.6.-*Dibenzanthracene* (60 mg./100 g. body weight). Animals killed 3 to 9 days later. The figure was low (mean 132) in 4 out of 5 females, and rather higher (mean 158) in 4 males.

(2) 2-*Acetamidofluorene* (40 mg./100 g. body weight). Animals (2 males, three females) killed 3 to 7 days later. The mean of the 5 concentrations was 126. (For further data about 2-acetamidofluorene see the following paper by M. E. Urquhart.)

(3) *Stilboestrol* (25 mg./100 g. body weight). One male, one female, killed 4 days later, gave 120 and 138.

(4) *Arachis Oil* alone (1 ml. 100 g. body weight) gave as mean figures 127 in 6 males and 123 in 7 females. These figures do not differ appreciably from those obtained when the above three compounds were injected in arachis oil.

In our earlier experiments on mice (Kennaway and Kennaway, 1944 ; Kennaway, Kennaway and Warren, 1944) arachis oil injected subcutaneously or intraperitoneally was found to lower the concentration of ascorbic acid in the liver by about 12 per cent, which change appeared to be due to enlargement of the liver.

*Arsenic.*

Arsenic is undoubtedly carcinogenic in man (for literature see Neubauer, 1947), but attempts by many workers to find any such effect in various species of animals have given almost wholly negative results. Guinea-pigs were given as high a concentration of arsenic in the drinking water as was tolerated over a long period. A solution (A) was made of 10 g. $As_2O_3$ and 10 g. $K_2CO_3$ in 1 litre, which is the same concentration of arsenic ($As_2O_3$) as that of Fowler's solution of the British Pharmacopoeia, of which the official dose for a man is 0·12 to 0·5 ml. three times daily. If one reckons 0·35 ml. as a mean dose, three times daily, a man (70 kg.) would take 10 mg. $As_2O_3$ per day.

At this rate a guinea-pig weighing 0·5 kg. would take $\frac{10}{140}$ mg.$= 0{\cdot}07$ mg. ; a dilution of Solution A was made containing this amount in 25 ml. (Solution B). The guinea-pigs received one feed daily of greenstuff which was not allowed to remain in the cage ; the dry diet of oats and bran then induced them to drink during the rest of the day. Solution B given as drink caused fatalities. The concentration was then reduced to $\frac{1}{4}$ of this amount, which, with occasional periods of rest, was tolerated. The ascorbic acid in the liver was estimated in 7 animals, of which the last 2 had been under treatment for 940 days ; ten females and 3 males lived for more than 635 days. All which died were examined, and no tumours of any kind were found.

*X-radiation.*

Eight guinea-pigs were exposed to X-rays (150r at half value layer, 250 kV. and 44.5 cm. focal skin distance). The dosage rate was approximately 68r/min.

Five animals died within a few months, the remaining three lived for over a year. No tumours of any kind were found.

*Riboflavin and ascorbic acid.*

In mice of some strains (Kennaway and Daff, 1946) the addition to a vitamin-free diet of riboflavin, either alone or together with casein, in amounts of 4 mg. per 100 g. dry diet, raised the ascorbic acid of the liver from the mean level given by the diet alone (187) to others (275, 256 mean 265), which are similar to the mean (275) given by yeast. In this instance, then, riboflavin was in this respect a complete substitute for yeast. Larger amounts of riboflavin (6·4 mg. or 8 mg. per 100 g.), either alone or together with thiamin or methionine, gave distinctly lower figures (234, 225, 212, mean 224). These results suggested the investigation described below.

Fifty-four male guinea-pigs were placed on a vitamin-free diet (B.D.H.), and to this was added riboflavin in amounts of 4 mg./100 g. (24 animals) or 8 mg./100 g. (6 animals); they were killed between the 3rd and 12th days (Table II and Fig. 1)

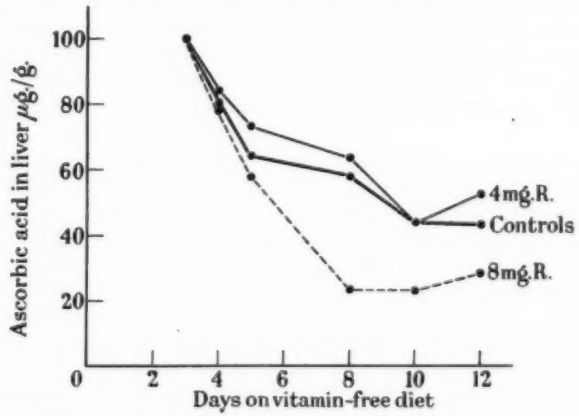


FIG. 1.—Ascorbic acid in Liver of Guinea-pigs (see Table II). Vitamin-free Diet with and without Riboflavin.

4 mg.R.: 4 mg. Riboflavin per 100g. Diet.
8 mg.R.: 8 „ „ „ „ „

and the ascorbic acid in the liver estimated. The graph shows that there was little difference between those receiving a diet with, or without, 4 mg./100 g. riboflavin, while 8 mg. riboflavin was associated with a more rapid diminution of ascorbic acid. The number of animals in this last series is small, but the uniformity of the curve suggests that the results are significant.

Thus both in the mouse and in the guinea-pig the smaller amount of riboflavin is associated with a larger amount of ascorbic acid. In the guinea-pig one might suppose that riboflavin increased the rate of consumption of the store of ascorbic acid. In the mouse the small amount of riboflavin can replace yeast or milk very efficiently as a supplement to a vitamin-free diet, and would seem therefore to promote the synthesis of ascorbic acid; possibly the larger amount of riboflavin

TABLE I.—*Comparison of Effects upon mean Concentration of Ascorbic Acid in the Liver in Guinea-pigs and Mice.*

| | | Guinea-pigs. | | Mice. | |
|---|---|---|---|---|---|
| | | Controls. | 1 : 2 : 5 : 6-dibenzanthracene. | Controls. | 1 : 2 : 5 : 6-dibenzanthracene. |
| Intraperitoneal injection | ♂ | 202 | 158 | 239 | 368 |
| | ♀ | 214 | 132 | — | — |
| | | | Stilboestrol. | | Stilboestrol. |
| | ♂ | — | 120 | 343,224 | 576,531 |
| | ♀ | — | 138 | — | |
| | | | 2-Acetamidofluorene. | | 2-Acetamidofluorene. |
| | ♂ | — | 121 | 297 | 371 |
| | ♀ | — | | 284 | 304 |
| Prolonged feeding | ♂ | — | 145 } 126 | — | — |
| | ♀ | — | 113 } | — | — |
| | | | Pregnancy. | | Pregnancy. |
| | | | 117 | 268 | 378 |

Numbers denote μg./g. wet weight.

TABLE II.—*Ascorbic Acid in the Liver of Guinea-pigs fed on Vitamin-free Diet with or without Riboflavin.*

| B.D.H. Vitamin-free Diet. | Number of guinea-pigs. | Days on diet before death. | Concentration of ascorbic acid in liver, μg./g. wet weight. Range. | Mean. | |
|---|---|---|---|---|---|
| Plus 4 mg. riboflavin per 100 g. | 4 | 3 | 92–118 | 98·8 | |
| | 4 | 4 | 61–120 | 84·1 | 85·5 |
| | 4 | 5 | 66– 88 | 73·5 | |
| | 4 | 8 | 50– 73 | 63·0 | |
| | 6 | 10 | 34– 57 | 44·0 | 52·8 |
| | 2 | 12 | 42– 61 | 51·3 | |
| | 24 | | | | |
| Plus 8 mg. riboflavin per 100 g. | 1 | 3 | — | 101·5 | |
| | 1 | 4 | — | 78·0 | 79·2 |
| | 1 | 5 | — | 58·0 | |
| | 1 | 8 | — | 24·0 | |
| | 1 | 10 | — | 22·8 | 25·1 |
| | 1 | 12 | — | 28·6 | |
| | 6 | | | | |
| Controls | 4 | 3 | 75–121 | 99·7 | |
| | 4 | 4 | 41–110 | 80·5 | 81·5 |
| | 4 | 5 | 31–103 | 64·3 | |
| | 4 | 8 | 52– 63 | 58·4 | |
| | 6 | 10 | 30– 56 | 43·8 | 48·5 |
| | 2 | 12 | 41– 45 | 43·2 | |
| | 24 | | | | |

brings into play also the increased consumption seen in the guinea-pig and hence the total of ascorbic acid is lessened. The question needs further inquiry with larger numbers of animals, as does also the resistance of the guinea-pig to carcinogenic agents.

## DISCUSSION.

Three agents which are carcinogenic in other species (2-acetamidofluorene in the rat, mouse, cat, rabbit and fowl: arsenic and X-radiation in man) have given negative results in regard to carcinogenesis in guinea-pigs.

Factors which in the mouse and rat increase the concentration of ascorbic acid in the liver (pregnancy; intraperitoneal injection of 1 : 2 : 5 : 6-dibenzanthracene, 2-acetamidofluorene, or stilboestrol, in arachis oil, and of the oil alone) have no such action in guinea-pigs and appear rather to produce a considerable diminution. Exact comparison of the ascorbic acid content of the organs of different series of guinea-pigs requires exact control of the intake of this compound which was not attempted in these experiments. These results as a whole suggest that the factors which increase the concentration of ascorbic acid in the liver of mice, and of rats, excite an increased demand for this compound to which animals capable of synthesising it respond by over-production, whereas in those lacking this power the store is depleted.

## SUMMARY.

The action of carcinogenic agents upon the guinea-pig is of interest because this species resembles man, and differs from the mouse, rat, and rabbit in its inability to synthesise ascorbic acid. Under the conditions stated, three agents, namely 2-acetamidofluorene, arsenic, and X-radiation failed to produce tumours in guinea-pigs. Compounds (2-acetamidofluorene, 1 : 2 : 5 : 6-dibenzanthracene, stilboestrol) of a class which in the mouse and rat increase the concentration of ascorbic acid in the liver have the opposite effect in the guinea-pig. The peculiar relation of riboflavin to the metabolism of ascorbic acid in the mouse, and in the guinea-pig, is compared.

We are indebted to Mr. J. S. Innes of the Department of Radiotherapy of this hospital for advice on X-radiation, and we wish to thank the British Empire Cancer Campaign and the Anna Fuller Fund for generous financial assistance.

## REFERENCES.

DAFF, M., HOCH-LIGETI, C., KENNAWAY, E. L. AND TIPLER, M. M.—(1948) *Cancer Res.*, **8**, 376.
KENNAWAY, E. L. AND DAFF, M. E.—(1946) *Brit. J. exp. Path.*, **27**, 63.
*Idem* AND KENNAWAY, N. M.—(1944) *Cancer Res.*, **4**, 704.
*Idem*, KENNAWAY, N. M. AND WARREN, F. L.—(1944) *Ibid.*, **4**, 367.
*Idem* AND TIPLER, M. M. (1947) *Brit. J. exp. Path.*, **28**, 351.
NEUBAUER, O.—(1947) *Brit. J. Cancer*, **1**, 192.

# THE METABOLISM OF 2-ACETAMIDOFLUORENE IN THE GUINEA-PIG.

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THE experiments described below form part of an investigation of the action of various carcinogenic agents upon guinea-pigs which was undertaken in view of (1) the resistance of the guinea-pig to many carcinogens and (2) the similarity of this species to man in its inability to synthesise ascorbic acid.

2-Acetamidofluorene when given with the food to rats produces malignant tumours of the liver, kidney, ureter, bladder, pancreas, acoustic duct, mammary gland, uterus and skin (Wilson, De Ed and Cox, 1941 ; Bielschowsky, 1944) and forms a derivative, the 7-hydroxy compound, which appears in the urine and can be demonstrated by means of a colour reaction with sodium nitrite (Bielschowsky, 1945). The experiments described below were designed to show, whether the guinea-pig reacted in these respects in the same way as the rat.

## EXPERIMENTS.

Guinea-pigs (both sexes) received daily about 30 g. of a mixture of oats (25 g.) and bran (75 g.) and 2-acetamidofluorene (0·07 g.) with fresh greenstuff and water, and at night were placed in a metabolism cage for collection of urine. The urine, of pH about 9, was stored with $CHCl_3$ ; amounts of 500 ml. were brought to pH 7 with hydrochloric acid and sodium acetate as buffer, and shaken with 4 lots of 200 ml. ether ; ethyl alcohol was used to break up emulsions. The ether extract was dried with $Na_2SO_4$, reduced to 50 ml., washed with dilute HCl, dried, and reduced to 15 ml.

To demonstrate the presence of the 7-OH compound about 5 ml. of the ether extract was evaporated to dryness, the residue taken up in glacial acetic acid, an equal volume of water and a few drops of 2N HCl added ; the liquid was cooled on ice ; addition of a few drops of cooled 0·1 per cent $NaNO_2$ produced a deep violet colour.

Amounts of 10 ml. ether extract were poured on to 50 ml. water and placed in the cold room for some weeks, when a pinkish precipitate appeared at the interface ; this material (m.p. 210°) was sublimed *in vacuo* ; the m.p. was then 227° and the m.p. of a mixture with a synthetic specimen was 229°.

An analysis (by Dr. Weiler) gave C 74·46 ; H 5·48, N. 5·59 per cent (calculated C 75·28, H 5·48, N 5·86 per cent). About 8 g. of the crude compound was obtained in 18 months, yielding 50 mg. of the pure compound but there was some decomposition during sublimation.

Feeding was continued for various periods up to 1471 days. When the animals were killed the ascorbic acid in the liver was estimated (see p. 606). No tumours of any organ were found.

The concentration of ascorbic acid in the liver of 5 of these, in $\mu g/g.$ wet weight, (148, 142, 119, 119, 100; mean of the females 145, mean of the males 113, mean of all 126) was about $\frac{2}{3}$ of the normal average amount; in the sixth animal the concentration (213) was at the normal level observed under the conditions of these experiments.

## DISCUSSION.

The guinea-pig thus produces no tumours in response to 2-acetamidofluorene given with the food, while similar treatment of the rat yields tumours of nine or more organs. Both species form the same 7-hydroxy derivative, but it was not possible to compare the quantities of it which they produce. The experiments provide another instance of the resistance of the guinea-pig to carcinogenic action.

A number of investigations from this laboratory, and from the Chester Beatty Research Institute (Daff *et al.*, 1948) have shown that the administration of carcinogenic compounds by any route to the mouse, and to the rat, is in almost all cases followed by an increase of the concentration of ascorbic acid in the liver. The behaviour of the two species, and of the guinea-pig, is therefore different.

## SUMMARY.

Administration of 2-acetamidofluorene to the guinea-pig with the food has no carcinogenic action such as is seen in the rat, but both species produce the 7-hydroxy derivative.

I am greatly indebted to Mr. Frank Goulden, A.R.I.C., for a synthetic specimen of the 7-hydroxy compound and for advice upon the technique of isolation.

I wish to express my thanks to the British Empire Cancer Campaign for a grant.

## REFERENCES.

Bielschowsky, F.—(1944) *Brit. J. exp. Path.*, **25**, 1.—(1945) *Biochem. J.*, **39**, 287.
Daff, M., Hoch-Ligeti, C., Kennaway, E. L. and Tipler, M. M.—(1948) *Cancer Res.*, **8**, 376.
Wilson, R. H., De Ed, F. and Cox, A. J.—(1941) *Ibid.*, **1**, 595.

# PREVALENCE OF SPONTANEOUS BENIGN AND MALIGNANT MAMMARY TUMOURS IN RIIIb MICE ACCORDING TO AGE AND PARITY.

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SPONTANEOUS benign epithelial tumours were found in the mammary glands of about 40 per cent and malignant in less than 2 per cent of breeders of 1 year of age and over in the RIIIb strain without milk-factor. Benign growths survived ovariectomy of their bearers for periods of at least 4 months, but their power to grow was limited. This was tested by grafting and subjection in new young homozygous hosts to normal hormonal stimulation, which was prolonged in unmated females and prolonged and increased in amount in breeders (Pullinger, 1952*a*, 1954). In this respect benign acinar growths of this strain differed from similar ones in C3H mice with milk-factor. The latter grew on grafting in the anterior chamber of the eyes of homologous and heterologous hosts (Browning, 1948). All palpable macroscopic infiltrating tumours found in RIIIb breeders grew continuously from the time they were first detected. This was probably at a later stage of development than those described by Foulds (1949) in C57 × RIII F1 hybrids associated with milk-factor, which showed interrupted progression of growth dependent on successive pregnancies. The malignant mammary tumours found in RIIIb females were invasive and all first grafts which were made grew in male hosts of the same strain. Previous observations on prevalence of mammary tumours of all kinds were based on figures for age and parity obtained in the course of breeding. According to the figures thus collected no mammary growths of any kind occurred under 12 months of age. Prevalence of benign growths appeared to increase with age and parity up to 19 months. Malignant tumours were found between 13 and 25 months, the majority over 18 months of age, none before 13 months and none in mothers of less than 3 litters. These earlier impressions have been retested and the observations extended in order to establish a basis for determining by substitution the kind and amounts of hormones responsible for the various pathological growths. Conclusions have been modified concerning benign proliferations showing squamous differentiation. These may form by 10 months of age and the vast majority are probably non-viable and non-absorbable. Prevalence of all types increased with parity. After 5 litters age alone did not increase incidence.

## METHODS.

Females of the pure line colony RIIIfB/Pu were set aside for observations on benign growths in groups of at least 20 to bear fixed numbers of litters before the age of nine months. Half the groups were killed at 11 months, thus allowing

time for post-partum involution, and half at 20 months, to observe the effect of ageing after comparable stimulation. Mice dying at 19 months were sometimes included because no increase had been found over this age. All nipple areas of all mammae were examined as previously described (Pullinger, 1947, 1949). Observations on the various groups were made concurrently over a period of 3 to 4 years. The groups chosen were mothers of 1 or 2 litters (Groups 1 and 2); 3 to 5 litters (Groups 3 and 4); 6 or more litters (Groups 5 and 6). Group 7 consisted of breeders or 6 or more litters which were never segregated, so that no limit was imposed on breeding. Groups 8 and 9 and 10 consisted respectively of nulliparous and unmated females. Breeders were drawn from first and subsequent litters (Table I).

For observation of incidence of malignant growths unlimited breeding was eventually allowed, but previously some limits were imposed with the aim of ensuring long life. Losses were severe from disease in some generations due to epidemics from *B. piliformis* infection; many breeders were taken from the colony for experiment; in 1950 the colony had to be divided. Further limitation of breeders included in this survey was due to requirements for age and parity. For these reasons the total number of breeders considered is comparatively small, 482 for 33 generations since cross-suckling. This figure includes 42 breeders of a cross within the strain (Table II). Most of these mice became very feeble at about 2 years of age and were then killed. Food and water were given *ad libitum*.

## RESULTS.

### *Benign tumours.*

Combined results for groups are shown in Table I. In Column C, Table II, the percentage number of breeders which can be expected to cease littering spontaneously after successive pregnancies will be found. Fig. 1 and 2 illustrate a diffuse purely acinar growth; Fig. 3 a compact lobular-acinar growth and Fig. 4 a mixed adenoacanthoma. All had survived ovariectomy. Inactive squamous epitheliosis have been described and illustrated previously (Pullinger 1949, Fig. 1 and 2).

*Groups* 1 *and* 2.—Among 26 breeders of 1 or 2 litters, 3 minute foci of inactive squamous epitheliosis were found in 3 mice at 11 months. Among 24 breeders killed at 20 months the mammae of 5 had a total of 6 benign growths, 4 of which were purely acinar.

*Groups* 3 *and* 4.—A mammary gland of 1 out of 24 breeders of 3 to 5 litters killed at 11 months had 1 inactive focus of squamous epitheliosis. The mammae of 12 out of 24 breeders killed at 20 months contained 19 benign growths of which 8 were purely acinar.

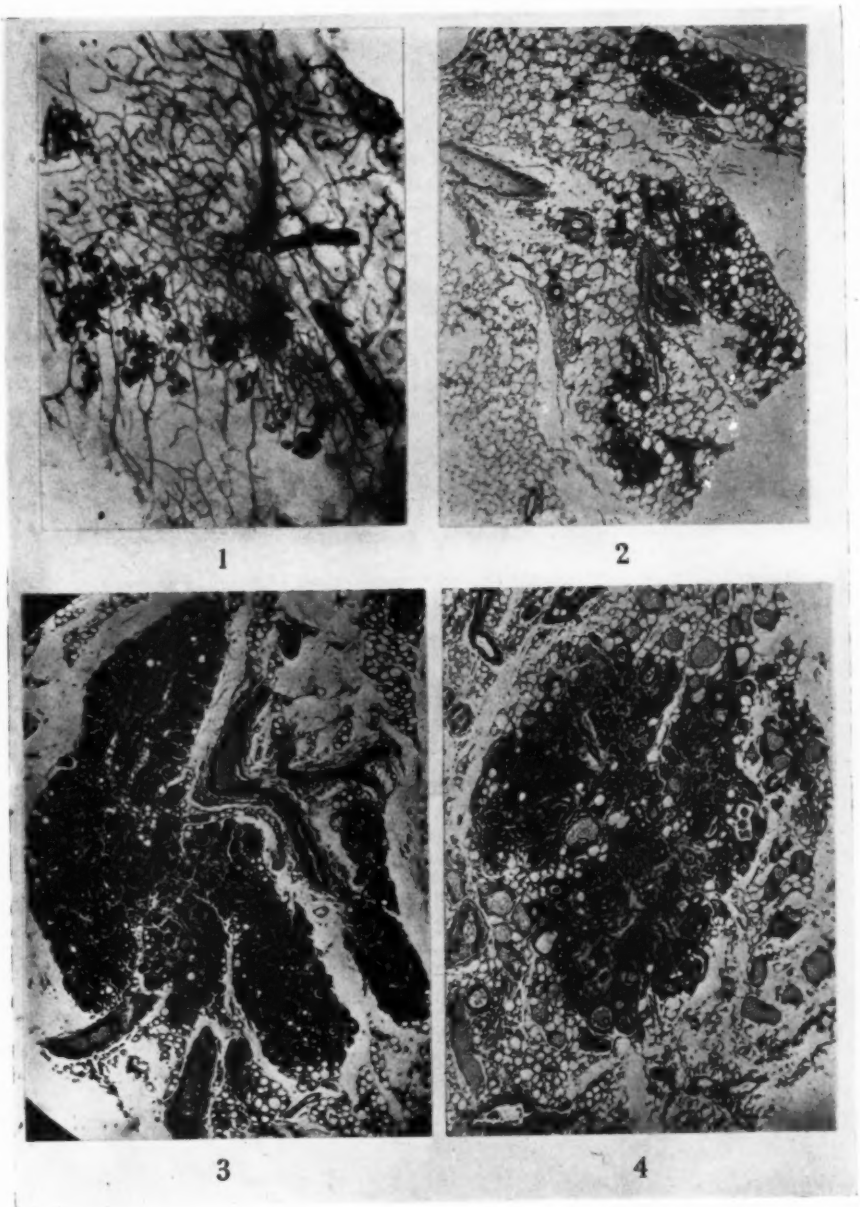
## EXPLANATION OF PLATE.

FIG. 1.—Bulk-stained specimen of a portion of a nipple region containing a diffuse acinar growth from a breeder ovariectomised at 20, and killed at 24 months of age. × 13.

FIG. 2.—Microscopic section of an acinar growth similar to that in Fig. 1. × 60.

FIG. 3.—Adenoma resembling normal lobule from a breeder ovariectomised at 16 and killed at 20 months of age. × 68.

FIG. 4.—A benign adenoacanthoma from the same mouse as Fig. 3. Foci of squamous epitheliosis are invariably present at the periphery of these growths. This makes possible their detection with a polariser. × 45.



1 2

3 4

Pullinger.

TABLE I.—*Comparison of Age, Parity and Incidence of Benign Mammary Growths in RIIIb Females.*

| Group. | Number of litters. | Conditions. | Age in months. | Number of mice. | Number of mice affected. | Number of benign growths. | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Acinar. | Inactive squamous. | Active squamous and mixed. | Total. |
| 1 | 1–2 | Segregated; killed at | 11 | 26 | 3 | 0 | 3 | 0 | 3 |
| 2 | 1–2 | ditto | 20 | 24 | 5 | 4 | 1 | 1 | 6 |
| 3 | 3–5 | „ | 11 | 24 | 1 | 0 | 1 | 0 | 1 |
| 4 | 3–5 | „ | 20 | 24 | 12 | 8 | 3 | 8 | 19 |
| 5 | 6 or more | Segregated at | 9 | | | | | | |
| | | Killed at | 11 | 21 | 10 | 0 | 10 | 9 | 19 |
| 6 | 6 or more | Segregated at | 9 | | | | | | |
| | | Killed at | 20 | 21 | 9 | 4 | 7 | 4 | 15 |
| 7 | 6 or more | Not segregated; killed at | 20 | 20 | 19 | 14 | 9 | 17 | 40 |
| 8 | Nulliparae | Killed at | 20 | 20 | 4 | 5 | 1 | 2 | 8 |
| 9 | Unmated | „ | 11 | 23 | 0 | 0 | 0 | 0 | 0 |
| 10 | „ | „ | 20 | 21 | 3 | 2 | 0 | 1 | 3 |

TABLE II.—*Comparison of Number of Litters and of Litter Sequence with Incidence of Mammary Carcinoma in 482 RIIIb Breeders.*

| Total litters. | Expected per cent self limited. | Mothers born in: First litters. Number of mothers. | First litters. Number with carcinoma. | First litters. Average age of survival in months. | Subsequent litters. Number of mothers. | Subsequent litters. Number with carcinoma. | Subsequent litters. Average age of survival in months. |
|---|---|---|---|---|---|---|---|
| 3 | 1 | 24 | 1 | 18·4 | 19 | 0 | 19·5 |
| 4 | 5 | 38* | 0 | 19·9 | 37 | 0 | 19·5 |
| 5 | 5 | 34 | 1 | 19·3 | 28 | 0 | 20·6 |
| 6 | 7 | 51 | 2 | 20·9 | 50 | 0 | 20·1 |
| 7 | 12 | 37 | 1 | 19·6 | 32 | 0 | 21·2 |
| 8 | 9 | 26 | 1 | 20·2 | 21 | 1 | 20·3 |
| 9 to 10 | 21 | 15 | 2 | 20·3 | 25 | 1 | 19·9 |
| 11 to 16 | 40 | 25 | 1 | 20·5 | 20 | 1 | 21·5 |
| | 100 | 250 | 9 | — | 232 | 3 | — |

* One which was transferred to an experiment developed a mammary carcinoma and was the only one which did so.

TABLE III.—*Analysis of* $\chi^2$.

| Source. | D.f. | $\chi^2$ | P. |
|---|---|---|---|
| Total | 7 | 35·9 | <·001 |
| Age | 1 | 7·7 | ·01–·001 |
| Parity | 3 | 19·8 | <·001 |
| Interaction | 3 | 8·35 | ·02–·05 |

*Groups* 5 *and* 6.—The mammae of 10 out of 21 breeders of 6 or more litters killed at 11 months contained 19 benign growths, none of which was purely acinar. The mammae of 9 out of 21 breeders killed at 20 months contained 15 benign growths, 4 of which were purely acinar.

*Group* 7.—Of 20 breeders mated for life and killed at 20 months, the mammae of 19 contained 40 benign growths, of which 14 were purely acinar.

*Group* 8.—The mammae of 4 out of 20 nulliparae killed at 20 months contained 8 benign growths, 5 of which were purely acinar.

*Groups* 9 *and* 10.—None of the mammae of 23 unmated females killed at 11 months contained any growths. The mammae of 3 out of 21 killed at 20 months contained 3 benign growths, 2 of which were purely acinar.

It appears from Groups 1 to 4, Table I, that ageing increased the number of mice affected with benign tumours, but this impression is not borne out in Groups 5 and 6. The same number of mice which had borne 6 or more litters were affected at 11 and at 20 months. Although total numbers are small, a statistical analysis was suggested by Dr. D. S. Falconer to answer the separate questions whether age and parity affect incidence and whether these two factors are independent of one another. The number of mice which could be expected to be affected in each group, if all were alike, was calculated from the proportion actually affected among the total examined in the whole table (omitting Groups 7 and 8, in each of which only one set of observations was made). The $\chi^2$ test was then applied to determine whether the differences between observed and expected were too great to have arisen from the chances of sampling. $\chi^2$ was calculated for each pair of observed and expected numbers and the 16 entries added together to give the total $\chi^2$. Similarly $\chi^2$ was calculated separately for age and for parity from the relevant figures. From Table III it will be seen that the total $\chi^2$ is significant, indicating that there are real differences between the groups, and that both age and parity affect incidence. An inconsistency appears, however, between the age and parity groups. Only in the 3 to 5-litter group is incidence strongly affected by age. In a total $\chi^2$ of 35·9, 7·7 is due to age and 19·8 to parity, leaving 8·35 with 3 for degree of freedom. This $\chi^2$ has a probability between 2 and 5 per cent of arising by chance and is not statistically significant. The results point to the existence of something else beside age and parity which has made the difference between Groups 3 and 4 greater than between other comparable groups.

*Malignant tumours.*

The earliest mammary carcinoma was found in a 13-month-old breeder. No malignant mammary growths occurred in 80 mothers of less than 3 litters, nor in 120 unmated females, nor in 40 nulliparae all of which lived to at least 13 months of age. Twelve mammary carcinomas occurred in 482 breeders which lived to 13 months or more and had each borne 3 or more litters. Of these, 1 occurred in a female derived from the cross within the strain. When subject to these limitations incidence was greater than 2 per cent. These breeders are arranged in Table II according to the number of pregnancies and according to their own birth sequence, whether born in their mother's first or in a subsequent litter. The females in the two groups were not always siblings, a precise comparison which is difficult to make in this strain owing to the capricious rearing

qualities of the breeders. To date no familial incidence has been found. From the table it may seem that the risk of developing mammary carcinoma was greater in breeders derived from first than from subsequent litters provided that the number of pregnancies was limited to less than 8; but these figures are not statistically significant. Of 12 mammary carcinomas in 482 breeders, 9 arose in 250 females from first and 3 in 232 females from subsequent litters. These 3 had borne 8, 10 and 14 litters respectively. Of breeders from subsequent litters there were 166 which had borne less than 8 litters. No mammary carcinoma occurred among these, but in the thirty-sixth generation (not included in Table II) one mammary carcinoma has arisen after 6 litters among 25 more survivors which had borne more than this number of litters. The figures above 3 litters do not support the view that incidence rises with increase in parity, as is often the case when milk-factor is present. Among 43 breeders which had 3 pregnancies only, there was 1 mammary carcinoma among 45 which had 11 to 16 pregnancies there were 2 carcinomas. No association was found between failure to rear litters, which is a common feature in this strain, and tumour incidence.

The average age at which malignant tumours appeared was 19·6 months. According to the classification of mammary tumours by Dunn (1953) 6 were type A adenocarcinomas. None could be classed as type B or C; 3 were adenoacanthomas. Two have been called squamous carcinomas because only after considerable search were acinar-like structures found and these did not resemble illustrations of molluscoid carcinoma. Grafts from the cellular shell, of one which was almost completely keratinised, grew in 11 out of 11 RIIIb females. One carcinosarcoma was found. Of the 12 carcinomas in the present survey 9 occurred in anterior nipple areas. Of the total spontaneous carcinomas seen to date all have been solitary, 17 out of 21 occurred in anterior nipple areas, 14 of which were in either second or third pairs. This distribution, though numbers are small, corresponds with the predominance found by Prehn, Main and Schneiderman (1954) of mammary carcinoma in anterior nipple areas of C3Hf females and of benign growths in RIIIb females (Pullinger, 1952*b*) in the absence of the milk-factor. When milk-factor was present in C3H females the former authors found a comparatively even distribution.

Microscopic invasive tumours have not been included owing to their debatable status. Their inclusion would double incidence.

## COMMENT.

Figures for parity provide only a rough guide to mammary gland stimulation but, as emphasized by Mühlbock and van Rijssel (1954) in relation to agent containing tumours, they may require to be known in order to evaluate tumour incidence. The view previously held (Pullinger, 1952*a*) that benign growths in agent free females do not appear to be due to excessive hormonal stimulation has been confirmed in unmated and nulliparous females and in breeders of 1 or 2 litters, but from the figures for unlimited breeding it is clear that increase in pregnancies increases the incidence and total number of benign growths of all kinds. Rapid breeding hastens the appearance of all except purely acinar proliferations. There is no evidence that this holds good for malignant mammary tumours in the RIIIb strain.

Purely squamous epithelioses were found in 6 out of 20 breeders of 6 or more litters at 10 months of age. This observation was missed before because few young and rapid breeders of 6 litters had been examined, nor was a polariser in constant use to reveal these keratinised structures. There is some evidence from transplantation experiments that the purely squamous-celled proliferations are non-viable. Even though associated with milk-factor in C3H mice, grafts of squamous metaplasia of a size comparable with those here described (0·1 to 0·2 cm.) never grew in anterior chambers in the eyes of other mice, whereas acinar foci (hyperplastic nodules with milk factor) grew in 4 out of 9 grafts (Browning, 1948, Table 2). Nevertheless the possibility that spontaneous or induced squamous carcinoma may arise in similar foci cannot be excluded. Spontaneous squamous carcinoma was, however, very rare, while squamous epithelioses are common. It is likely that the vast majority are dead, non-absorbable structures. Mitoses have never been found in them.

As in the previous survey, no purely acinar growths were found in young females under 1 year of age whether mated, regardless of the number of litters, or unmated, in contrast with the large number and high incidence found in young virgin females even as early as 9 months, when milk factor is present (Pullinger, 1952*a*). Attention was first drawn by Huseby and Bittner (1946) to the reduction in " hyperplastic nodules " after cross suckling females of some high cancer lines on low mammary cancer strain mothers. Fekete, Little and Richardson (1952) failed to find them in 62 young virgins of 8 to 9 months old of the C3Hb/Fe strain. Mühlbock, Tenbergen and Rijssel (1952) found none in 47 virgin females less than 15 months of age in the DBAb strain. When present in large numbers in young females whether mated or not, purely acinar nodules appear to be a reliable guide to the presence of agent, but their absence does not exclude this possibility, as is known from their absence from strain A.

## SUMMARY.

1. The incidence of benign mammary growths in RIIIb females increased with parity but not with age after the fifth litter. Six or more pregnancies acted alone in increasing the incidence of growths associated with squamous change in young mice to that attained in older animals which had borne fewer litters. The greatest incidence and total number of all varieties of benign growths were seen in females subjected to unlimited breeding. Benign tumours were distributed equally between females derived from first and from subsequent litters.

2. No purely acinar growths were found before 12 months of age either in multiparae or in unmated females.

3. No spontaneous mammary carcinomas arose in unmated females or in breeders of less than 3 litters or less than 13 months of age. Incidence did not increase thereafter with parity.

It is a great pleasure to acknowledge the help I have received from Dr. D. S. Falconer concerning the statistical significance of these results and to whom I am indebted for Table III.

The work was done while holding the Alice Memorial Fellowship and with a grant for expenses from the Imperial Cancer Research Fund.

## REFERENCES.

BROWNING, H. C.—(1948) *J. nat. Cancer Inst.*, **8**, 173.
DUNN, T. B.—(1953) 'Physiopathology of Cancer.' New York (Paul B. Hober), p. 123.
FEKETE, E., LITTLE, C. C. AND RICHARDSON, F. L.—(1952) *Cancer Res.*, **12**, 219.
FOULDS, L.—(1949) *Brit. J. Cancer*, **3**, 345.
HUSEBY, R. A. AND BITTNER, J. J.—(1946) *Cancer Res.*, **6**, 240.
MÜHLBOCK, O. AND VAN RIJSSEL, T. G.—(1954) *J. nat. Cancer Inst.*, **15**, 73.
MÜHLBOCK, O., VAN EBBENHORST TENBERGEN, W. AND VAN RIJSSEL, T. G.—(1952) *Ibid.*, **13**, 505.
PREHN, R. T., MAIN, J. M. AND SCHNEIDERMAN, M.—(1954) *Ibid.*, **14**, 895.
PULLINGER, B. D.—(1947) *Brit. J. Cancer*, **1**, 177.—(1949) *Ibid.*, **3**, 494.—(1952*a*) *Ibid.*, **6**, 69.—(1952*b*) *Ibid.*, **6**, 78.—(1954) *Ibid.*, **8**, 161.

# THE EFFECT ON PREVALENCE OF MAMMARY TUMOURS OF AGEING ALONE AND OF OESTROGEN SUBSTITUTED AFTER OVARIECTOMY IN RIIIb BREEDERS.

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OBSERVATIONS on spontaneous benign mammary growths in intact RIIIb breeding females lacking milk-factor suggested that their prevalence increased with parity rather than with age. Up to 5 litters the effect of age appeared to be strong. Only 1 out of 24 females was affected at 11 months and 12 out of 24 at 20 months but this impression was not supported by counts after 6 or more litters. When 21 breeders had borne at least 6 litters the number affected at 11 and at 20 months of age was 10 and 9 respectively (Pullinger, 1955). It was probable that any effect of age on incidence was due to a continuation of oestrus cycles when breeding was stopped after the smaller number of litters. To test this conclusion mice that had borne various numbers of litters were ovariectomised at 9 to 10 months before viable growths were expected to appear. One group had no further treatment; oestrone was applied to another. All were killed at 20 months and the mammae examined. It was found that ageing alone of the ovariectomised mice resulted in a fifteen-fold reduction in incidence of viable benign growths. No malignant tumours appeared after ovariectomy alone but the number of breeders used was too small to judge whether carcinoma had been suppressed. When oestrone in doses of 0·1 to 0·15 mg. was substituted after spaying, incidence, total number and variety of growths, both benign and malignant, was that to be expected in intact multiparae segregated at the same age.

## METHODS.

The mouse strain, breeding, examination of mammae and ovariectomy were the same as previously described (Pullinger, 1947, 1949*a*). Only pure line progeny were used. Breeders were segregated from males 1 month before spaying. Age groups used were 9 to 10 and 11 months old breeders. The last was needed to find out if benign growths could be prevented by ovariectomy at 11 months and if not, whether they would survive for the remainder of the experimental period. When oestrone was substituted it was applied in a 0·01 per cent solution in acetone to the skin of the back from which hair had been clipped. Doses of 0·05 ml. were applied once weekly for 20 to 30 weeks, the actual total dose varying from 0·1 to 0·15 mg. Four months were allowed to elapse before the mammae were examined to ensure regression of any physiological response.

## RESULTS.

Before stating results it can be confirmed that no restoration of oestrus cycles, nor adrenal hyperplasia or tumours have ever been seen in mice of this strain

after ovariectomy at puberty. None has been spayed in the first week after birth. The results of 2 groups, 3 to 5 litters and of 6 or more litters by 9 months of age correspond with those in intact mice in Table I of the previous paper (Pullinger, 1955). The mammae of 16 ovariectomised breeders of 3 to 5 litters were free of benign growths of any kind when examined at 20 months. A mammary gland of 1 out of 17 mothers of at least 6 litters contained 1 fibrous adenoma. In 45 intact breeders 21 were affected with benign growths ; in the two ovariectomised groups together one breeder out of 33 was affected, a fifteen-fold reduction in incidence after ovariectomy.

When oestrone was applied for 6 months after ovariectomy in total doses of 0·1 to 0·15 mg. the mammae of 14 out of 31 breeders of 3 to 8 litters, examined at 20 months, contained benign growths. Of these 11 were purely acinar, 6 were adenoacanthomas. At 18 months one of these treated breeders developed a carcinoma—a malignant adenoacanthoma. This female was derived from a first litter and had herself borne 4 litters. The incidence of benign growths was restored by oestrone to about the number and variety to be expected in intact breeders. The single carcinoma did not represent any increase above the usual incidence.

When ovariectomy was delayed until 11 months and the mammae examined at 20, 6 out of 13 breeders of 6 or more litters were affected with benign growths, 2 of which were viable adenoacanthomas and 4 were foci of purely squamous proliferation. These observations suggest that 11 months is critical for development of viable benign growths, that is to say some at least cannot be suppressed when ovariectomy has been delayed to that age. They prove also that, having formed, viable growths can persist without ovarian hormones for 9 months. Previously the survival time after ovariectomy had not been tested for longer than 4 months.

In some earlier experiments which were prematurely ended by an epidemic of Tyzzer's disease, 8 breeders had been ovariectomised immediately after their fourth litters at 4 to 6 months of age. After intervals which varied from 6 to 11 months, these 8 were treated with the same dosage of oestrone. Three developed purely acinar growths found at 20 months. These few observations support the view that benign viable growths which appeared after ovariectomy and oestrone treatment were not preformed and thereafter merely maintained. All the substitution experiments suggest that potentially benign, neoplastic foci, previously determined by multiple pregnancies, were promoted or developed by oestrogen. This action is analogous to promotion in experimental carcinogenesis of skin warts (Friedewald and Rous, 1944).

In other unpublished experiments a dose of oestrone 10 times larger than that used here failed to induce mammary carcinoma or to cause malignant change in benign growths in 37 breeders which had been ovariectomised at 16 months, treated to 20 and allowed to survive. This dose was sufficient to provoke lobular-alveolar development and milk-like secretion (Pullinger, 1949*b*).

## COMMENT.

In comparing the effects of age and of oestrogen substituted after ovariectomy the inactive squamous epithelioses have been ignored because it is probable that they are dead structures which have no further significance. They are, however, indicators of the earliest tendency to pathological proliferation.

At one time, during the course of these observations, carcinoma had appeared only in females derived from first litters. An association was then sought between liability to carcinoma and the possible influence of the X-zone of the adrenal overlapping in time the early development of the mammary gland. Extracts of X-zone from young mice were injected daily for 3 days into pregnant multiparae from the fourteenth to sixteenth days of pregnancy judged by the vaginal plug test. One only out of 18 female progeny developed a mammary carcinoma after unrestricted breeding. Later, carcinoma was found in breeders from litters other than the first and an association could no longer be suspected.

Owing to scarcity of malignant mammary tumours in the RIIIb strain, and thus the need for greater numbers of animals, the methods here used to relate tumour incidence and hormone action could not be applied.

SUMMARY.

1. The incidence of benign mammary growths in old female mice was decreased fifteen-fold by ovariectomy after breeding. Malignant growths were too rare to apply the same methods to test the effect of age after ovariectomy on their incidence.

2. When oestrone was substituted after ovariectomy the proportion of benign and of the only malignant mammary tumour which occurred was comparable with that in intact breeders in old age. Benign were not converted into malignant growths by oestrone in the doses used.

The work was done while holding the Alice Memorial Fellowship and with a grant from the Imperial Cancer Research Fund.

## REFERENCES.

FRIEDEWALD, W. F. AND ROUS, P.—(1944) *J. exp. Med.*, **80**, 101.
PULLINGER, B. D.—(1947) *Brit. J. Cancer*, **1**, 177.—(1949*a*) *Ibid.*, **3**, 494.—(1949*b*) *Lancet*, ii, 823.—(1955) *Brit. J. Cancer*, **9**, 613.

# THE EARLY EFFECTS OF 9 : 10-DIMETHYL-1 : 2-BENZANTHRACENE ON MOUSE SKIN, AND THEIR SIGNIFICANCE IN RELATION TO THE MECHANISM OF CHEMICAL CARCINOGENESIS.

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It is known that most skin carcinogens, applied to the body surface, are eliminated from the body in the course of a week or two. The resultant tumours take several months to appear. This would seem to point to some sort of irreversible action on one or more of the " permanent " structures (*e.g.*, dermis, vascular or nerve supply, etc.) or on the treated field as a whole, rather than on individual epidermal cells which are subject to loss by attrition and otherwise. When it was generally believed that carcinogens were only effective if applied at intervals over a long period, it was possible to accept the hypothesis of a direct attack on the cell, though even then it was difficult to understand why the later attacks were so much more effective than the early ones. But when it was established by Berenblum and Shubik (1947) that a single application of a potent carcinogen was adequate if followed up by a co-carcinogen (croton oil) which does not itself produce many tumours, it became necessary to postulate a two-stage action if the direct cellular hypothesis was to survive.

Experiments from this department (Billingham, Orr and Woodhouse, 1951 ; Marchant and Orr, 1953, 1955) have shown that if the actual carcinogen-treated epidermis is transplanted to an untreated site on the other side of the body, it does not develop tumours even if subsequently treated with croton oil. The same applies to thin Thiersch grafts ; in order to transplant the carcinogenetic field it is necessary to use thick Thiersch or whole-skin grafts. On the other hand, if the carcinogen-treated area is deprived of its original epidermis, and resurfaced with untreated epidermis, tumours appear in the usual incidence.

Such findings strongly support the view that the essential action of the carcinogen is not on the epithelial cell itself, but there remained the possibility that the effective epithelium was contained in the deeper parts of the hair follicles. If so, it is strange that skin tumours induced in the ordinary way only rarely show trichoepitheliomatous structure (though the skin tumours of mice treated over a wider body field with methylcholanthrene in oil not infrequently show follicular or sebaceous differentiation). Though many workers have already investigated the role of hair follicles in experimental carcinogenesis, it was thought worth while to see what happens to the follicles after a single treatment with 9 : 10-dimethyl-1 : 2-benzanthracene, which was not one of the carcinogens included in a previous histological survey (Orr, 1938). The results, which form the subject of the present communication, were more dramatic than was anti-

cipated, in that the epithelial elements of the existing hair follicles were completely destroyed. Their manner of reconstitution has important bearings on the general problems of skin carcinogenesis already referred to.

## EXPERIMENTAL MATERIAL AND CLINICAL OBSERVATIONS.

The mice were albinos from the general laboratory stock (random breeding). They were kept in metal boxes (5 to a box and sexes segregated) and fed on the standard laboratory diet of rat cube, with water *ad lib.* 9 : 10-Dimethyl-1 : 2-benzanthracene (DMB) 0·5 per cent in acetone, was applied to the skin (unclipped) in the region of the right scapula with a capillary pipette in amount sufficient to cover an area of about 2 sq. cm. before evaporation of the solvent took place. The mice were thereafter examined daily, and representative animals killed each day for histological examination of the skin. The skin for histology was removed in one piece including the whole of the treated area and a border of 0·5 to 1 cm. of normal skin around it ; the deep surface was applied to filter paper to keep the skin flat, and the skin was fixed in 4 per cent formaldehyde saline. Paraffin sections were stained with Ehrlich's haematoxylin and eosin, Weigert's haematoxylin and van Gieson, and Lawson's stain for elastic tissue.

In a small preliminary group of 6 mice to determine the time of epilation, this was found to be complete over the treated area in 6–7 days. In view of the unexpected result of histological examination of these mice (*vide infra*), the main series of 70 mice was histologically sampled from the first day onwards ; this series was started in 2 batches with a 2-day interval between, to obviate difficulties of serial sampling over the week-ends.

Slight thinning of the fur was suspected in some mice after 2 days, and about a third of them showed definite commencing epilation at 4 days. These mice showed complete epilation of the treated area at 6 days, when the others showed lesser degrees. Epilation was complete in practically all the mice at 7 days. Thereafter there was considerable variation in their rate of recovery. In general, it seemed that those mice which took longer to become epilated, recovered hair more rapidly. On the day following epilation, the bald surface was usually covered by a sticky eczematous exudate. This might be replaced a day later by a matt surface representing regeneration of epidermis ; or the exudate might persist, undergoing coagulation with the formation of a stiff scab. Histological examination showed that this difference was due to the presence of greater damage to the dermis in the latter case (*vide infra*). At 6 days after epilation (= 12 or 13 days after DMB treatment) there was great variation in the picture seen, some mice having a complete matt epithelial covering with fine new hair, while others showed depressed ulcers having fibrinous exudate in their floors and marginal ingrowth of epithelium. About two-thirds of the mice showed macroscopic re-epithelialisation of the epilated surface 12 days after epilation (= 18 or 19 days after DMB), but in a few ulceration persisted.

The 42 mice remaining after 24 days were painted twice a week with croton oil (0·5 per cent in acetone), in order to establish that one application of DMB was an effective carcinogen under the conditions of the experiment. The first papilloma appeared 16 days after croton oil treatment started, and when the experiment was terminated by killing the mice 160 days after the initial DMB treatment 22 of them had developed tumours, of which a few were malignant.

The tumours were sometimes multiple, but only two of them might have been regarded as originating outside the treated area.

Observations were also made using weaker solutions (0·125 per cent and 0·05 per cent) of DMB. The rate and incidence of epilation were much less, *e.g.*, with 0·125 per cent DMB about one quarter of the mice showed minor degrees of epilation at 7 days, and over half at 10 days when only a few were completely epilated. The results correspond, as does their histology, with those obtained with the less potent carcinogenic hydrocarbons, and it is not necessary to go into detail, except to say that they indicate that the qualitative effects of weaker solutions of DMB seem to be in line with those of the more fully studied methylcholanthrene, benzpyrene, etc.

## HISTOLOGY.

The pilot series (epilated and killed at 6–7 days) showed complete coagulative necrosis of the entire treated superficial epidermis and hair follicles (Fig. 1). The detail of the histological findings is incorporated in the description which follows.

One day after DMB. The epidermis shows a slight excess of superficial keratin and in places is slightly separated from the dermis. There is some enlargement of the epithelial cells and a slight tendency to stratification (up to 3 layers). The hair follicles are normal, but the bulbs are very darkly stained with cells packed together. Slight oedema and fibrillation of superficial margin of dermis.

Two to three days after DMB. Some intensification of above changes. A proportion of the sebaceous glands have disappeared.

Four days after DMB (no clinical epilation). Some parts of epidermis show complete coagulative necrosis; in other parts some of the nuclei still stain normally; keratinised flake over entire treated surface. Hair follicles similarly variable, some completely destroyed, some only affected at their mouths. Sebaceous glands almost completely gone. The dermis, subcutis, deep fascia and mammary fat are oedematous and lightly infiltrated with inflammatory cells. Outside the treated area there is stratified squamous hyperplasia (? regenerative) of the epidermis.

Five days after DMB (no clinical epilation). All the superficial epidermis and the majority of the hair follicles are necrotic (Fig. 2); in a few hair follicles a small number of cells still stain, but it is doubtful if they are viable; sebaceous glands destroyed. Ulceration of dermis at one point; superficial dermis fibrillary; oedema and inflammatory infiltration of deeper layers down to mammary tissue. Squamous hyperplasia of the epidermis at edges of treated area. The changes are much greater than macroscopic examination would have suggested.

Six days after DMB (complete clinical epilation). The epidermis is destroyed over the treated surface, and replaced by protein exudate and debris, except for one small central area where it is hyperplastic, stratified and bizarre. The epithelial components of the hair follicles are completely necrotic, but their dermal outline remains intact. At the edges of the lesion, the epidermis is stratified and pleomorphic squamous, and the hair follicles replaced by pegs of undifferentiated squamous epithelium. Oedema and inflammatory infiltration of dermis-subcutis and deep fascia.

Seven days after DMB (1 day after complete clinical epilation). Thick crust of keratin, fibrin and inflammatory cells on surface. The epidermis and hair

follicles are completely gone in the centre; the dermal outline of the follicles is preserved and the spaces contain inflammatory cells and macrophages. Oedema and cell infiltration of deeper structures as before. At the edge, there is stratified regenerative hyperplasia of the epidermis, and in a very few hair follicles bizarre squamous ?regenerating epithelium.

Seven days after DMB (the day of complete clinical epilation). Changes as in the preceding, but already the squamous epithelium has started to spread in from the edge, underneath the separated crust of necrotic original epidermis and hair follicles. It consists of a few layers of rather flat cells, with small pegs of undifferentiated regenerative epithelium in the dermal baskets of the destroyed hair follicles (Fig. 3). The arrangement of elastic tissue round these baskets leaves no doubt as to their nature.

Nine days after DMB (3 days after epilation). Scab on surface, epidermis gone. Hair follicles also destroyed, but dermal outline still intact in many of them. Some ulceration of dermis, and severe inflammation of the deeper layers with early fibrosis of subcutis. At the edges, regenerative squamous hyperplasia of epidermis, spreading in under scab, and inserting undifferentiated epithelial pegs into hair follicle baskets.

Nine days after DMB (2 days after epilation and showing some recovery of fine downy hair). Damage less, and recovery greater, than in preceding. The epidermis is practically intact, but is all squamous with various degrees of stratification (normal mouse epidermis being unstratified cuboidal epithelium). The hair follicles are much less numerous in the treated area than in the surrounding untreated zone, but are cellular; some of them are replaced by undifferentiated squamous epithelium, but some contain hairs, even down in the subcutis. There are no inflammatory changes in the subcutis. This animal is unique in the present series, in that there was no evidence of massive epidermal necrosis. The picture was such as might have been expected after treatment with a weaker carcinogen, and is possibly just an example of biological variation. Another possibility, arising from the presence of hair follicles in the subcutis, is that this mouse was in a hair growth phase at the time of treatment, when according to Borum (1954) the damage from DMB is less than in the resting phase of the hair cycle.

Ten to twenty-three days after DMB. During this period there was much variation in the rate of recovery processes, depending mainly on the extent of damage to the dermis. In a few cases the superficial dermis became separated as a slough incorporated with the necrotic epidermal material and exudate, and regeneration of the epidermis and hair follicles from the edges took place below the deep edge of this slough as it separated (Fig. 4 and 5). Resurfacing with squamous epithelium was complete within 12 days of DMB treatment (5 to 6 days after complete epilation) when damage to the dermis was not sufficient to destroy its architectural pattern; on the other hand, when severe sloughing of the dermis had occurred, resurfacing might still be incomplete at the end of the period of observation. Regeneration of hair follicles and sebaceous glands was similarly affected; this is not surprising in view of the fact that sloughing of the dermis implied loss of the pre-existent dermal baskets in which reconstitution of the hair follicles took place in the less severely damaged skins.

Allowing for these differences in tempo, the histological material available makes it clear that the process of reconstitution of the epidermis and hair follicles

follows a standard pattern. All the new epidermis was derived from ingrowth, or inward spread, from the untreated normal epidermis at the edge of the lesion. No contribution was made by the mouths of hair follicles as they were all destroyed and did not reappear until the ingrowing epidermal covering had reached their site. Observations made under the present conditions left no doubt that the new hair follicles were differentiated *de novo* from the regenerated epidermis.

The sequence seems to be as follows. Pegs of undifferentiated squamous epithelium grow out from the deep surface of the new epidermis, into the pre-existing dermal baskets if still available. These pegs increase in size and undergo central keratinisation ; sometimes the keratin becomes laminated and tube-like as if a rudimentary hair was being attempted ; sometimes small cysts are formed (Fig. 11). At times the appearances may resemble the cell nests of squamous carcinoma, but this is only a histological similarity as they do not persist After a few days the pegs grow downwards through the dermis and into the subcutis, developing at the same time typical darkly-staining hair bulbs at their deep extremity ; the new follicles then become canalised and hair production restarts. It is uncertain what happens to the keratinous cysts ; the present findings suggest that they participate in the formation of hair follicles, because it has not been possible to find any other way by which they are eliminated. Sebaceous glands are reformed more slowly, the first evidence having been seen at 13 days after DMB treatment (7 days after epilation) ; the first traces of them appear in the mid-dermal region of the new hair follicle, sometimes before the development of a hair.

The foregoing statements are mainly based on the stage of organisation reached by hair follicles in different parts of the section. It was found that in general those at the periphery of the treated area, having started regeneration earlier, showed more advanced maturation than those in the centre. Fig. 6 to 11 exemplify the findings in two animals. It is of interest to note also that the newly-differentiated follicles may be in the growth phase, while those in the surrounding untreated skin are in the resting phase (Fig. 9 and 10).

Attention has already been drawn to the great quantitative variation in damage to the dermis and subcutis. The depth to which changes were found was unexpected. The present observations were not continued long enough to determine final effects on these tissues. Sometimes the fibroblastic reaction in the fascia of the panniculus carnosus was surprisingly brisk. Subcuticular scarring occurred in some of the animals. The texture of the dermal collagen had not returned to normal at the end of these observations, even though inflammation and oedema had subsided.

## DISCUSSION.

The results of the present experiment leave no reasonable doubt that under its conditions hair follicles and sebaceous glands can be regenerated from the superficial epidermis. This is at variance with traditional views on the subject, which up till quite recently have not even been controversial. Wolbach (1951), in his careful study of the hair cycle of the mouse in relation to carcinogenesis with benzpyrene and methylcholanthrene, stated without qualification that new follicles do not form from the epidermis, under any circumstances, after the original post-natal formation has been completed. With these carcinogens, and with weaker solutions of DMB, reparative hyperplasia of the hair follicles occurs.

without a previous stage of complete necrosis, so that there is justification for his statement in the facts available to him.

The experiments of Bredis (1954) however, in which full thickness wounds of the skin of rabbits were prevented from contracting by metal rings, seemed to show conclusively that the scar epithelium was capable of redifferentiating into hair follicles and sebaceous glands.

Gillman, Penn, Bronks and Roux (1955) give a documented review of the present status of the relation between hair follicles and tumours. They believe that the original hair follicles and sebaceous glands of mice are usually completely destroyed by chronic treatment with methylcholanthrene, and that if new ones arise they do so *de novo* from epidermal pseudo-pegs. They also suggest that carcinoma may result if in epidermal regeneration there is failure to eliminate intra-dermal epithelial pegs or to organise new hair follicles from them In the present experiment, as in previous studies, one is struck by the histological similarity of some of the regenerative follicular changes to the cell nests of squamous carcinoma ; similar structure is illustrated in Fig. 5 and 6 of the paper of Liang and Cowdry (1954) on changes after a single painting of methylcholanthrene.

Andreasen and Engelbreth-Holm (1953) found that one application of 0·5 per cent DMB in benzene gave a much higher yield of papillomas if the hair cycle was in the resting phase than when it was in the growth phase. Borum (1954) in following up this work with a modified technique, confirmed it, and further showed that much greater immediate damage was inflicted in the resting phase, the time for epilation being about half that required in the growth phase. The present author observed that the comparative depilatory effects of polycylic hydrocarbons run parallel with their carcinogenic potency (Orr, 1938). There is much evidence, therefore, of the vulnerability of hair follicles to carcinogenic agents but it must be stressed that the effects are those of damage rather than primary stimulation.

There is nothing in the present observations which is incompatible with the suggestion already mentioned of Gillman *et al.* (1955) regarding the possible histogenesis of tumours, or with that of Wolbach (1951) if it is qualified to admit the possibility of regeneration or attempted regeneration of hair follicles from the superficial epidermis. In Wolbach's view, carcinomas arise in papillomas, and papillomas arise from hair follicles which have lost their papilla rest cells, necessary for the organisation of the mature follicle. If this is so, it must of course be accepted that the capacity to produce papilla rest cells is not confined to the superficial dermis, because although hair regeneration is quicker when the original dermal baskets are retained, it can occur more slowly after sloughing of the superficial dermis. The idea that epithelial tumours of the skin arise from frustrated attempts to form hair follicles is also supported by the marked infrequency of such tumours in non-hair-bearing sites, *e.g.*, the palms of the hands and soles of the feet, but it is not the same thing as the statement that the tumours arise from hair follicles themselves.

The present results assist in the interpretation of the information available from the transplantation experiments of Billingham, Orr and Woodhouse (1951) and Marchant and Orr (1953, 1955). It is now shown that, at least under certain circumstances, all the epithelial components of carcinogen-treated skin may be derived by ingrowth from the surrounding epidermis. If the treatment with carcinogen leads to the irreversible intra-cellular change of " initiation ", so

that latent cancer cells are already present, it might be expected to follow that the transplanted epidermis from a carcinogen-treated site should be competent to produce tumours. Grafting experiments have shown that this does not happen even after prolonged co-carcinogenic treatment of the graft, and the general conclusion now seems to be justified that while carcinogens have primary effects on both epithelium and stroma, the permanent necessary effect is that on the stroma. Once such changes are present, it might be that any epithelium growing on the surface and subjected to intrinsic or extrinsic stimuli to proliferation, might respond by producing a tumour. This implies that the tumour cell first emerges shortly before the appearance of the tumour itself, and not at the time the epithelium is in immediate contact with the chemical carcinogen.

In the present experiments, is it reasonable to believe that the epithelium of the new surface, regenerating inwards from the untreated skin outside, has not been directly attacked by the carcinogen? The experiments of Woodhouse (1954, 1955) show that protein-binding of polycyclic hydrocarbons occurs whether they are carcinogenic or not, and that the maximum concentration of bound DMB coincides with the period of damage to the epidermis (*i.e.*, before and during the time of necrosis). There is, however, still a minute amount of bound, and a somewhat larger amount of free, hydrocarbon in the necrotic superficial layer when ingrowth starts to take place beneath it. The proliferating cells thus come in contact with DMB, but having regard to its insolubility in water and the absence of sebaceous glands to provide a fatty solvent for it, there are great difficulties in accepting that it can be transported into the cytoplasm of the regenerating cells.

It might be argued that it is impossible to limit the DMB to the ostensibly " treated " area, even with a highly volatile solvent. This cannot be denied, but there should be taken into consideration that (1) the most accurate delineation of the treated area is obtained from the effects, especially epilation which is generally sharply circumscribed with little evidence of a fade-out at the periphery, (2) the great majority of subsequent tumours arise well within the treated area, and (3) normal sebaceous glands are found immediately outside the treated area, at a time when they are completely destroyed within it.

## SUMMARY.

Outbred mice received a single application of 9 : 10-dimethyl-1 : 2-benzanthracene (0·5 per cent in acetone). This results in epilation of the treated area in 6–7 days, and is an effective carcinogenic stimulus if supplemented later by co-carcinogenic treatment.

Histological examination showed necrosis of the epidermis and other epithelial structures of the treated skin, starting on the 4th day and complete by the 6th–7th day.

Thereafter, the epidermis was regenerated by ingrowth from the untreated periphery, and hair follicles and sebaceous glands were reconstituted by differentiation *de novo* from the new epidermis.

These results are discussed in relation to the results of grafting experiments previously reported. The available evidence gives no support to the view that the carcinogenic hydrocarbons cause a direct intracellular pre-neoplastic change in the epithelial cell itself. It seems therefore that the essential change or changes must occur in the deeper tissues of the carcinogenetic field.

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## REFERENCES.

ANDREASEN, E. AND ENGELBRETH-HOLM, J.—(1953) *Acta path. microbiol. scand.*, **32**, 165.
BERENBLUM, I. AND SHUBIK, P.—(1947) *Brit. J. Cancer*, **1**, 383.
BILLINGHAM, R. E., ORR, J. W. AND WOODHOUSE, D. L.—(1951) *Ibid.*, **5**, 417.
BORUM, K.—(1954) *Acta path. microbiol. scand.*, **34**, 542.
BREEDIS, C.—(1954) *Cancer Res.*, **14**, 575.
GILLMAN, T. PENN, J., BRONKS, D. AND ROUX, M.—(1955) *Brit. J. Cancer*, **9**, 272.
LIANG, H. AND COWDRY, E. V.—(1954) *Cancer Res.*, **14**, 340.
MARCHANT, JUNE AND ORR, J. W.—(1953) *Brit. J. Cancer*, **7**, 329.—(1955) *Ibid.*, **9**, 128.
ORR, J. W.—(1938) *J. Path. Bact.*, **66**, 495.
WOLBACH, S. B.—(1951) *Ann. N.Y. Acad. Sci.*, **53**, 517.
WOODHOUSE, D. L.—(1954) *Brit. J. Cancer*, **8**, 346.—(1955) *Ibid.*, **9**, 418.

## EXPLANATION OF PLATES.

FIG. 1.—Skin of mouse 7 days after DMB (day of complete epilation). Epidermis and hair follicles completely necrotic; sebaceous glands destroyed. Dermis oedematous. Inflammatory cell infiltration of subcutis. × 145.

FIG. 2.—Five days after DMB (*not* clinically epilated). Epidermis and hair follicles completely necrotic. Dermis oedematous. Slight cell infiltration of subcutis. × 145.

FIG. 3.—Seven days after DMB (day of complete epilation). Field from periphery of treated area. Layer of new squamous epithelium which is spreading in from the untreated margin, beneath the necrotic remains of the original epidermis and hair follicles. Note undifferentiated epithelial pegs in the dermal baskets of the hair follicles. × 135.

FIG. 4.—Ten days after DMB. In the central part (left of picture) of the treated area there has been damage to the dermis (part of the separated slough of epidermis and superficial dermis can be seen at top of picture). At the right can be seen a hair follicle with sebaceous gland (outside the treated area), and the regenerating epithelium can be seen spreading in, with downgrowths into the hair follicle baskets of undifferentiated epithelium. × 45.

FIG. 5.—Fifteen days after DMB. Normal skin on left. Epidermal regeneration, beneath dermal slough on right. Undifferentiated epithelial pegs on right (most recent regeneration); keratinised epithelial cysts in middle (more advanced regeneration). × 50.

FIG. 6, 7, and 8.—Twelve days after DMB. Different fields from same section.

FIG. 6.—Normal skin outside treated area. × 100.

FIG. 7.—Peripheral part of treated area. Epidermis stratified. Regenerated hair follicles show bulbs, and foci of sebaceous cells, but are not yet canalised. *Cf.* Fig. 8. × 100.

FIG. 8.—Central part of treated area. Epidermis stratified. Intradermal epithelial pegs show keratinised cyst formation, but are not yet recognisable as hair follicles. *Cf.* Fig. 7, where the stage of regeneration is more advanced. × 100.

FIG. 9, 10 and 11.—Twenty-three days after DMB. Different fields from same section.

FIG. 9.—Normal skin outside treated area. × 100.

FIG. 10.—Peripheral part of treated area. Hair follicles completely regenerated, but only traces of sebaceous gland tissue. Note, however, that follicles are in the growth phase (bulbs down into the subcutis) as compared with untreated skin at edge (Fig. 9). × 100.

FIG. 11.—Central part of treated area. *Cf.* Fig. 10. Reconstitution of regenerated hair follicles much less advanced. × 100.

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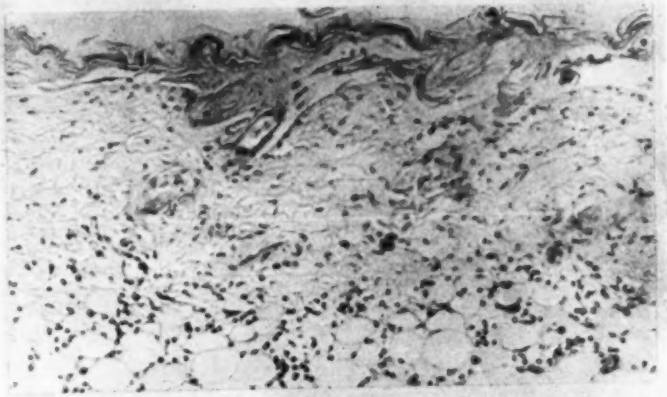

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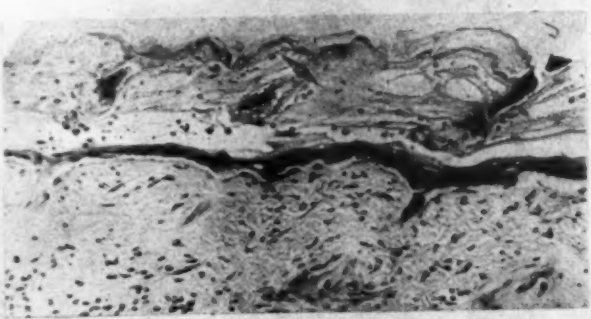

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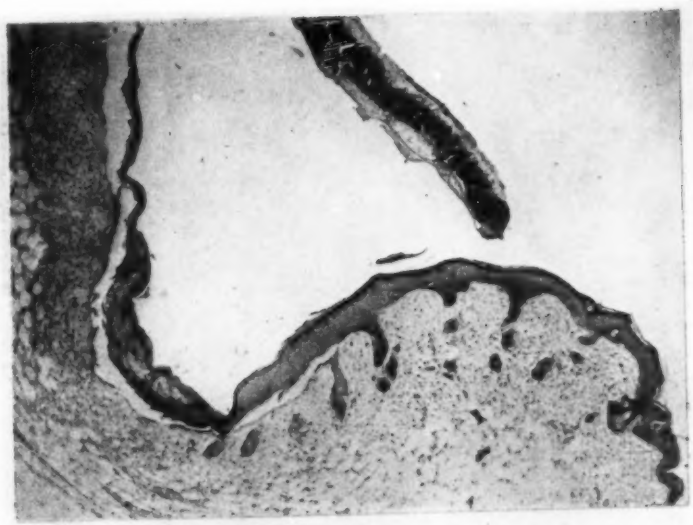

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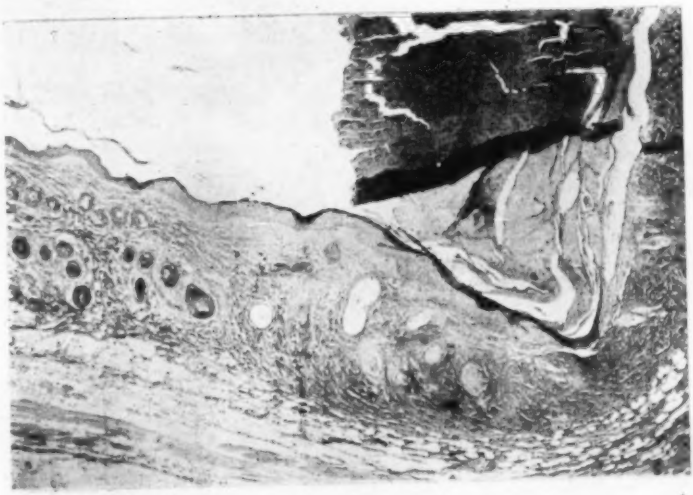

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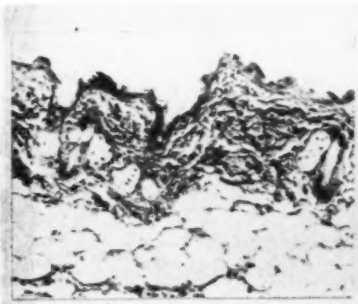

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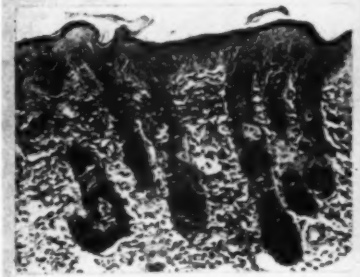

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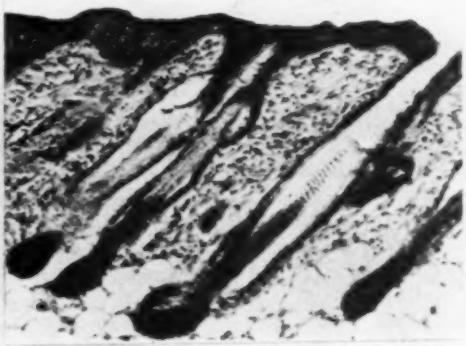

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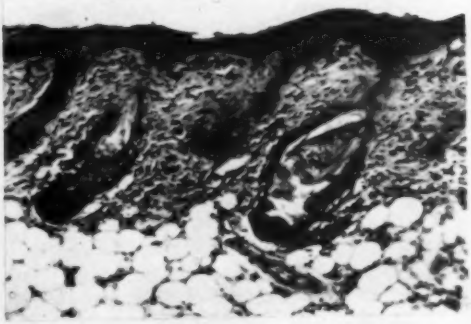

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# THE PRODUCTION OF A VARIETY OF SKIN TUMOURS IN RATS WITH 2-ANTHRAMINE, AND A COMPARISON WITH THE EFFECTS IN MICE.

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THE experiments to be described form the first stage in a projected attack by experimental methods on the old problem of the nature and origin of the rodent ulcer. Purely observational methods are by no means entirely exhausted, but it seems unlikely that they will ever supply a complete explanation (some account of the literature and of my own views in this direction will be found elsewhere (Lennox and Wells, 1951 ; Lennox, 1954.) An experimental attack is obviously called for, but so far has been held up for lack of a suitable experimental situation. The present experiments do no more than establish that a suitable experimental situation now exists and is worth exploiting ; but numerous incidental points of interest make it seem worth reporting at this stage.

Bielchowsky (1946) reported that 4 of 18 rats painted with 2-anthramine (2-amino-anthracene) developed rodent ulcers. At the time when the present experiments were started I believed that this had been the only experimental production known, but two others have since come to my notice. Bachmann *et al.* (1937) reported two rodent ulcers produced in the rat by methyl cholanthrene : and Dr. M. H. Salaman (personal communication) has seen one rodent ulcer in a mouse fed arsenic and painted with croton oil. It seemed necessary first of all to confirm Bielchowsky's (1946) results, using larger numbers of rats, to make certain that the tumours could be produced in other strains of rats, and to establish a baseline for further work. An experiment with 2-anthramine on mice was later undertaken to elucidate some points arising from the first experiment.

## EXPERIMENT I : RATS.

*Animals.*—Forty male rats of our own albino stock, which has a high proportion of Wistar blood, were used. They were 33–37 days old when painting commenced.

*Carcinogen.*—2-Anthramine (m.p. 240–242° C.) was prepared for me by Dr. W. Klyne by reduction of 2-aminoanthraquinone (Ruggli and Henzi, 1930). A saturated solution in acetone (1 per cent approximately) was used. The croton oil mixture was made by similarly saturating a 1 per cent v/v solution of croton oil in acetone with the dye. 2-Anthramine stains the skin deep yellow, and its distribution can be readily followed. Solutions darken with time, but this can be retarded by keeping them in the refrigerator ; they were never used more than three weeks after preparation.

*Application.*—The lumbar skin between the iliac spines was used throughout. Depilation and a dropping pipette were used at first, but it soon appeared that hair

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actually protected the dye from being licked off, and depilation was thankfully abandoned. The solutions were then applied beneath the hair with a tuberculin syringe and a hypodermic needle whose blunted point was held in contact with the skin. The skin remained stained for at least two days after each application.

*Dosage.*—Solutions were applied twice a week throughout. Beginning with a single drop (0·02 ml.) dosage was raised rapidly to 0·1 ml. on the 27th day, kept at this level till the 120th day, and then raised steadily till by the 300th day it was 0·3 ml. The area of skin stained thus expanded as the rat grew, reaching a maximum diameter of about 40 mm. with the highest dose. All rats received 2-anthramine alone at first ; from the 102nd day half the rats received the 2-anthramine-croton-oil mixture instead.

*Toxicity.*—At the lower dosages the rats remained in excellent health. At the highest level (3 mg. approximately twice weekly) some loss of weight occurred, but no deaths could be attributed directly to the 2-anthramine. Post-mortems on all rats except three were performed, and liver and lungs examined histologically ; no specific lesions were detected.

*Collection of tumours.*—At approximately fortnightly intervals all rats bearing tumours 2 mm. or more in diameter had these excised under ether with a substantial margin of non-tumour-bearing skin. (Because of the difficulty in recognizing minute tumors—especially those in the dermis—the " time of appearance " of tumours is shown as that of this first biopsy. The error involved is rarely much more than a week.) Painting was continued till a second tumour appeared, when the rat was killed. (These second tumours are referred to later as " recurrences ", though they were most often new tumours.)

## *Results.*

One rat died of a respiratory infection on the 312th day without tumours. The remaining 39 all bore tumours in the treated area, the first appearing on the 231st day and the majority between the 290th and 370th days. There were no tumours of untreated skin, but three rats bore non-cutaneous sarcomata. " Recurrent " tumours were available for study in 32 rats. Of the remainder 5 died from various causes soon after the first tumours had been excised, and two were killed on the 448th day (at the end of the experiment) without sign of recurrence. In most cases tumours were multiple and of several sorts (Fig. 2).

*Varieties of tumour.*—The varieties of tumours seen are listed in Table I, and briefly annotated below.

### *Non-cutaneous tumours.*

Two rats developed fibrosarcomata of the jaw, one of the lower and the other of the upper (Fig. 1). Both grew rapidly, and both animals had to be killed. The rats often nuzzle the painted areas on themselves and others, and these tumours could be ascribed to direct action of the carcinogen on the gums. One rat developed a massive tumour of the peritoneum, which became covered with rounded pedunculated firm whitish nodules ; it was cellular, with a slightly whorled structure, and might be mesotheliomatous. This strain of rats has been used in Professor E. J. King's silicosis experiments in this department for some time ; at least a hundred rats have survived for over a year and no spontaneous tumours have been

TABLE I.—*Numbers of Rats Bearing Tumours of Various Kinds.*

| | |
|---|---|
| Non-cutaneous tumours | 3 |
| Sarcoma of jaw | 2 |
| Sarcoma of peritoneum | 1 |
| Cutaneous tumours | 39 |
| Sarcomata | 35 |
| Epithelial tumours | 28 |
| Squamous tumours | 22 |
| " Ordinary " squamous carcinoma | 14 |
| Squamous carcinoma " ex cyst "* | 7 |
| Squamous keratosis | 3 |
| Squamous papilloma | 1 |
| Rodent group tumors | 15 |
| " Ordinary " rodent ulcers | 12 |
| Rodent ulcer " ex cyst "* | 2 |
| Nodular rodents | 4 |
| Sebaceous group | 10 |
| Hair follicle cysts | 2 |
| Sebaceous carcinoma " ex cyst "* | 2 |
| Sebaceous carcinoma | 7 |

* I.e., arising from cystic structures of hair follicle origin, resembling the sebaceous cysts of man.

observed, so a relation of all three tumours (produced just within the year) to the carcinogen must be regarded as probable.

*Cutaneous sarcomata.*

These are much the commonest tumors, being seen in 35 rats and being often multiple. In the early stages they form dome-shaped purplish elevations somewhat reminiscent of the human dermatofibrosarcoma protuberans (Pack and Tabah, 1951) to which they present some interesting analogies. Microscopically they appear as malignant-looking proliferations of the dermis, usually of the deepest part (Fig. 2). They grow rapidly and often ulcerate ; on the deeper aspect invasion of the panniculus muscle is usual, but no fixation to the deeper tissues was seen. On three occasions recurrent sarcomata were allowed to grow to a considerable size (up to 60 mm. diameter) until ulceration made it necessary to kill the rats, but no metastases were seen. In the present context these tumours are chiefly a nuisance, as they interfere with the production of the epithelial tumours in which I was chiefly interested.

*Squamous tumours.*

These are the commonest epithelial tumours (22 rats). They have been classified into carcinoma (invasive), keratosis (premalignant—equivalent to the " senile " keratosis of man) and papilloma, according to a nomenclature advocated elsewhere (Lennox, 1954). The meaning of " ex cyst " in the table will be explained later, under " Hair follicle proliferation ". Though the squamous carcinomata were clearly invasive, occasionally invading the panniculus, and they would be classified without hesitation as malignant if met in routine human biopsy reporting, none metastasized.

*Rodent group.*

Fifteen rats in all showed tumours of this group. Twelve rats bore rodent ulcers of the familiar human variety, some very small, but all readily recognizable as

such ; the largest were 10 to 12 mm. in diameter and quite characteristic (Fig. 3). Four nodular sub-epidermal tumours thought to correspond to the " nodular rodents " (epithelioma adenoides cysticum or solitary Brooke's tumour, more or less) were also seen. The two " ex cyst " lesions of the table will be explained later. This whole group obviously requires more detailed description, but it is intended to defer this until a larger number of cases has become available.

*Sebaceous group.*

The sebaceous carcinomata resembled squamous carcinomata in histology but contained variable numbers of sebum cells, whose lipid content could be readily stained in frozen sections. Most of them lay in the superficial dermis, without clear connection to surrounding structures ; from their site an origin from hair follicle necks or sebaceous glands could be suspected, but was not proved. Two (*vide infra*) arose from " sebaceous " cysts. In all cases the peripheral cells of each tumour island consisted of squamous cells, sebum cells occupying the centre ; the latter rarely formed a continuous mass, but were dotted singly among squamous cells.

*Hair follicle proliferation.*—The cycle of changes leading to the production of tumours in the walls of cysts apparently similar to the human " sebaceous " cyst is of some interest. Hair follicle enlargements and irregularities are present in a large proportion of specimens. The follicle widens greatly and becomes filled with an irregular mass of keratin. The sebaceous glands vanish (this phenomenon is, of course, well known in mice painted with methyl cholanthrene ; Montagna and Chase, 1950). The hair follicle origin of these lesions is demonstrated especially clearly by those examples (Fig. 4) in which the hair matrix cells can still be recognized in the base of the lesion by their small size and basiphilia. Keratin-filled cysts are present in a few rats ; it may be presumed, though direct evidence is lacking, that they arise from these overgrown hair follicles. Like human sebaceous cysts, they show no sebaceous differentiation. Some irregular epithelial proliferation is seen in the walls of most of these cysts (Fig. 5). This goes on to actual tumour formation more often than not, the tumours being of all the three main varieties. (*a*) *Squamous carcinoma " ex cyst "* (7 cases) : This could be anything from a localized area of malignancy in the wall of a cyst to complete replacement of the cyst by a solid mass of epithelium whose shape alone gave evidence of its origin (fig. 6). (*b*) *Rodent " ex cyst "* (2 cases) : Buds of rodent-ulcer epithelium arose from the outer aspect of crypts of this kind exactly as they arise in the more usual type of tumour from the undersurface of an intact epidermis. (*c*) *Sebaceous carcinoma " ex cyst "* (2 cases) : The two largest and best developed sebaceous carcinomata both appeared to arise from cysts (Fig. 7).

*Effect of croton oil.*—Croton oil was used with two ideas, neither successful : (*a*) that since its action is largely superficial, it might increase the proportion of epidermal tumours at the expense of those of the dermis—a hope not fulfilled, as the proportions of different kinds of tumours in the two groups were almost identical. (*b*) That it might either speed up the appearance of tumours or increase the yield. The yield was unaffected, and the rate of appearance of tumours actually slowed. The first tumour appeared in the 2-anthramine group in the 231st day, the first in the 2-anthramine-croton-oil group on the 297th day, and a similar lag was maintained throughout. The mean time of appearance in the 2-anthramine

group was 313 days (standard error 35·6 days) and in the mixture group 353 days (standard error 34·1 Days); the difference between the means is 40 days and its standard error 11·2 days.

## EXPERIMENT II: MICE.

The mouse experiment was designed to answer three questions. First, whether 2-anthramine was carcinogenic for the skin of mice (reported experiments with it refer to rats only). Second (supposing the answer to the first question to be affirmative) whether Bielchowsky's (1946) success in producing rodent ulcers was due to the use of an unusual carcinogen or an unusual animal. Third, whether the anti-carcinogenic action of croton oil could be demonstrated and studied in mice.

*Material and methods.*—Male albino mice of a "Glaxo" strain were used, 30 days old at the commencement of painting.

*Carcinogen.*—Most of the possible mixtures of 2-anthramine and croton oil—in strengths of 1 per cent, 0·3 per cent and 0 per cent in acetone were used, on groups of five mice each. In two groups the two agents were used alternately instead of being mixed together. Owing to the inconclusive nature of the results it does not seem worth wasting space to give exact details of dosage rates.

*Application.*—The acetone solutions were painted on the undepilated skin with a calibrated brush, the mid-dorsal region being used.

*Dosage.*—Approximately 0·05 ml. was painted on twice weekly.

*Toxicity.*—A pilot experiment showed that mice appeared healthy and gained weight normally when painted with this dose of 1 per cent 2-anthramine (0·5 mg.) thrice weekly for a month. The longer term painting (twice weekly at the same dose) however proved less satisfactory, chiefly because of the appearance of mouse ectromelia; this infection was widespread in our strain of mice but remained silent under most conditions. It appeared to some extent in all groups of mice and was severe in most, even those painted with croton oil alone. No evidence of more direct toxic effect of the 2-anthramine was obtained.

### *Results.*

Including the pilot experiment, 47 mice in all were painted, and 42 were still alive on the 140th day, when the first tumour appeared. Other mice bore tumours on the 147th day, 215th and 229th days. Because they were found to be a source of salmonella infection to other mice they all had to be killed on the 265th day: careful examination of the painted areas then showed small lesions in four further mice, of which three were histologically little more than areas of hyperkeratosis, but one was a definite squamous papilloma. All the four mice originally bearing tumours had survived to this date; histological examination of these showed that two were papillomata (multiple in one case) and two showed local invasion and must be called carcinomata. All the tumours were squamous in type.

There were thus eight tumours in all, of which two were malignant; one was seen in each of eight of the nine groups into which the rats had been divided. This suffices to show that 2-anthramine is a weak carcinogen for rat skin, but the long period of painting necessary and low yield of tumours discouraged attempts to study the croton oil effect further by this method.

## DISCUSSION.

The original and ultimate object of these experiments is, as has already been said, an experimental attack on the nature and origin of the rodent ulcer. This first generation of rats has sufficed to show that in the 2-anthramine painted rat we have at least a possible subject for such experiments. It is far from the ideal subject ; the long duration of painting necessary and the appearance of so many other tumours at the same time are great disadvantages. But nothing else offers, and it is hoped that the investment of a moderate number of years' work may produce some results.

One small fact emerges even at this stage. Rodent ulcers have been produced from skin without sweat glands. Hence they cannot be tumours of sweat glands. There have, to be sure, been no serious attempts to suggest that they *are* sweat gland tumours, but it is nevertheless something to be certain on this point. It is of interest in the same connection to note that whereas Lennox and Wells (1951) found some evidence of fluid secretion in 57 per cent of human rodent ulcers, it was seen in only 1 of the 18 rat tumours (and not very certainly in that). This can only be partly accounted for by the small size of the rat tumours, and may be regarded as indirect evidence for Lennox and Well's view that the fluid they saw was sweat, and that its appearance in the human tumours was at least in some way related to the fact that the epidermis from which those tumours arose had been able in the foetus to give rise to sweat glands.

### *The croton oil effect.*

The action of croton oil in delaying the onset of tumours was totally unexpected Several possible hypotheses may be examined.

(a) *Species difference.*—The cocarcinogenic action of croton oil well established in the mouse (Berenblum, 1941, 1947 ; Salaman, 1952) may not apply in the rat, in which in the absence of proof it is possible, though unlikely, that it has an anti-carcinogenic action. Testing with other carcinogens in the rat might settle this.

(b) *Overstimulation.*—Even with effective cocarcinogens excessive doses may reverse the effect, especially if the skin is damaged by a strong irritant. The croton oil, however, produced no acute skin lesions and did not accelerate depilation.

(c) *Chemical union.*—To save labour in a prolonged experiment the 2-anthramine and croton oil were applied mixed. It is possible that the two react together so as to reduce the effective dose of 2-anthramine. (Croton oil is a complex mixture and its chemistry appears to be obscure.)

At one stage the last of these hypotheses seemed very probable, for it was noted that the darkening of acetone solutions of 2-anthramine which occurs with time is considerably accelerated by admixture with croton oil. Dr. W. Klyne suggested that a study of the absorption spectra would be a suitable way to test this. Miss Irene Broadbent and Miss Brenda Cavanagh independently compared the spectra of fresh 2-anthramine solutions with 2-anthramine-croton-oil mixtures which had stood in a cupboard at room temperature for 2–3 weeks. They found no significant difference between them in the region 260–700 m$\mu$. This makes it improbable that loss of 2-anthramine occurs in these solutions. The third hypothesis is therefore unlikely to be true.

The mouse-painting experiment was designed to explore this problem further. However, as has been indicated, the results were unhelpful in themselves and not such as to encourage further work in this direction. For the present therefore these curious results with croton oil remain unexplained.

*Resemblances between skin tumours in man and the rat.*

The same carcinogen applied in the same way to different individuals produces different tumours, and may even excite them at closely related points in the one individual. There is here a problem, or rather a whole group of problems well worth study. In the great rush of work on carcinogenesis which followed Kennaway and his school's discovery of the carcinogenic hydrocarbons, the very existence of this has been obscured by the accident (not wholly an accident perhaps, for part of the convenience of the object lies in this simplicity) that the mouse skin, by far the most convenient test object for most experiments on carcinogenesis, produces for all practical purposes but the one tumour, the squamous carcinoma and its benign precursors. The skin of man, however, produces a very much greater variety of tumours, both spontaneously and in response to recognised carcinogens. Other species for which data are available (Willis, 1953 ; Head, 1953 ; Cotchin, 1954) produce also a considerable variety of skin tumours. Hence it would appear that the mouse, however excellent an object for some experimental purposes it may be, gives nevertheless a very inadequate idea of the processes of neoplasia in the skin. The rat is probably more representative of mammals in general, and certainly at least presents a reasonably close resemblance to man in this respect.

The similarity of the majority of the rat tumours seen in these experiments to human lesions needs no detailed discussion. Sebaceous carcinomata similar to those seen here are, of course, rare in man (I have myself yet to see one), but are well enough authenticated (Warren and Warvi, 1953). Squamous carcinoma (Caylor, 1925 ; King, 1947) and rodent ulcer (Caylor, 1925) have been recorded arising in man from " sebaceous " cysts. The sarcomata can be equated with dermatofibrosarcoma protuberans (Pack and Tabah, 1951) resembling it strongly in its local malignancy without metastasis and in its clinical appearance during the early stages ; the more rapid growth in the rat is, however, reflected in a more variable and in general more anaplastic histology and in a greater tendency to ulceration than are ordinarily met with in the human lesion. Some major groups of human tumours—notably the melanomata and hidradenomata—are unrepresented and the relative proportions of those tumours which are represented is widely different from those seen in man : but in general it seems reasonable to say that we have here the closest parallel to the diverse tumours of the skin of man yet produced experimentally.

Carcinogens would seem in general merely to accelerate the production of tumours to which the species is naturally prone. Certainly no recognized carcinogen in man has ever been recorded as producing a tumour which cannot also appear spontaneously—whatever, in this context, " spontaneously " may mean. The tumours we have produced in the rat ware probably those which any carcinogen acting at the same site would produce (Bachmann *et al.*, 1937) and are probably also those which would occur naturally. It is a curious thought that the easiest way to determine the " natural " incidence of tumours in any tissue of any species may be to expose it to some artificial carcinogen.

SUMMARY.

1. Forty rats were painted with 2-anthramine on the lumbar skin. Skin tumours appeared in 39, and sarcomata elsewhere appeared in three of these 39.

2. The skin tumours were dermal sarcomata (35 rats) and epithelial tumours (28 rats), the latter being classifiable into the squamous group (22 rats), rodent ulcer group (15 rats) and sebaceous group (10 rats).

3. Tumours of several kinds were seen arising from " sebaceous " cysts in 11 rats.

4. Croton oil appeared to have an anticarcinogenic action.

5. In mice 2-anthramine was only weakly carcinogenic, producing squamous lesions, two of them carcinomata, in 8 out of 47 mice painted.

My thanks are due to Professor J. H. Dible for his continued encouragement and to Dr. W. Klyne, Miss Irene Broadbent and Miss Brenda Cavanagh for biochemical assistance, to Dr. Jean Duncan Taylor for painting the rats during my absences, to Mr. J. Griffin for the histological preparations and to Mr. C. A. P. Grahame for the photomicrographs.

## REFERENCES.

BACHMAN, W. E., COOK, J. W., DANSI, A., DE WORMS, C. G. M., HASLEWOOD, G. A. D., HEWETT, C. L. AND ROBINSON, A. M.—(1937) *Proc. Roy. Soc.* B., **123**, 343.
BERENBLUM, I.—(1941) *Cancer Res.*, **1**, 807.—(1947) *Brit. med. Bull.*, **4**, 343.
BIELCHOWSKY, F.—(1946) *Brit. J. exp. Path.*, **27**, 54.

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## EXPLANATION OF PLATES.

FIG 1.—Fibrosarcoma of the upper jaw of a 2-anthramine-painted rat. A cross-section of the whole upper jaw region. H. & E. × 2. At higher powers the tumour was seen to be a fibrosarcoma with no trace of bone formation.

FIG. 2.—Squamous carcinoma (left) and dermal fibrosarcoma (right) arising in close proximity in the 2-anthramine painted skin of a rat. Both tumours have invaded the panniculus carnosus. This has been completely destroyed within the main fibrosarcoma nodule, but to the right of it sarcomatous proliferation can be seen both superficial and deep to surviving muscle. H. & E. × 2.

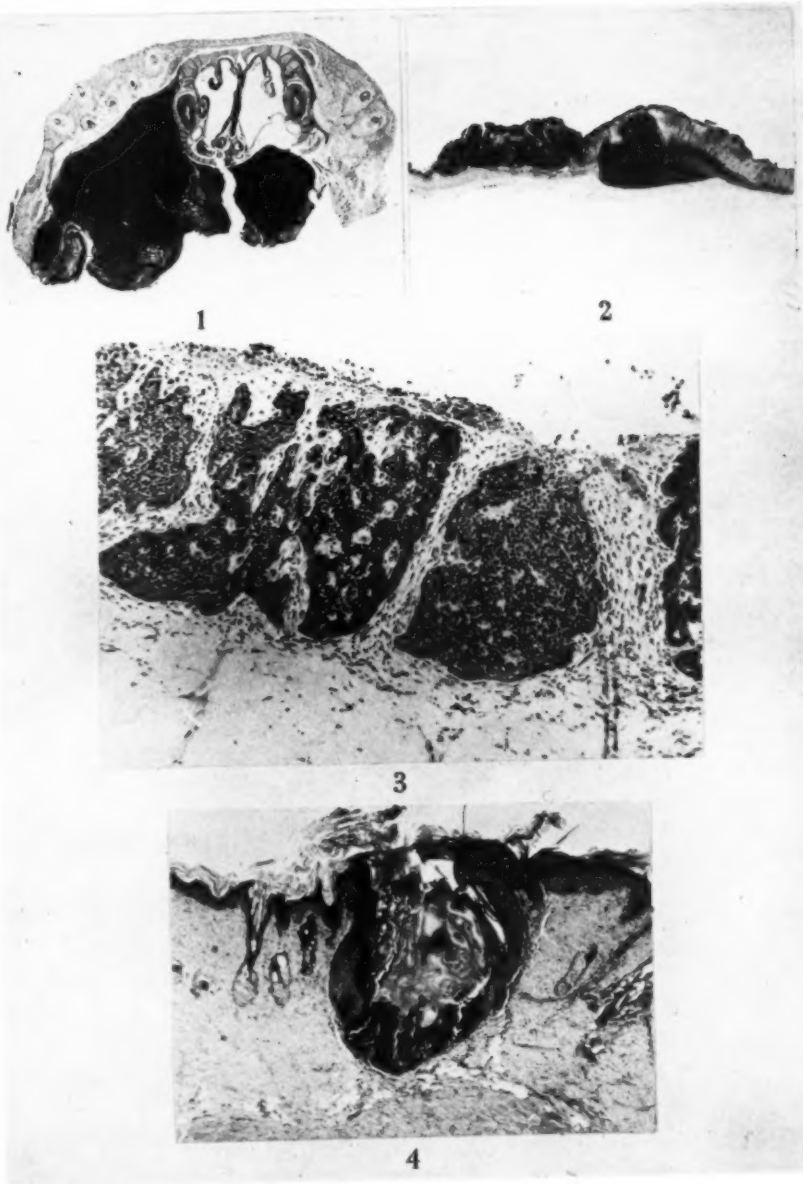
FIG. 3.—Rodent ulcer arising in the painted skin of a rat. H. & E. × 92.

FIG. 4.—Hair follicle overgrowth in painted rat skin. The follicle has lost its sebaceous glands and is plugged with keratin. There is hyperplasia both of the sheath of the follicle and the more basophilic matrix cells at the base. H. & E. × 34.

FIG. 5.—" Sebaceous " cyst with early epithelial hyperplasia in the direction of squamous carcinoma. The cyst has been filled with keratin. Presumably such structures are derived from hair follicles like those seen in Fig. 4. H. & E. × 43.

FIG. 6.—Squamous carcinoma " ex cyst." This consists entirely of (malignant) squamous epithelium, but its shape suggests an origin from a hair follicle rather than the surface epithelium, and a cyst like that of Fig. 5 and 7 may well have been present at an earlier stage. Note the invasion of the panniculus carnosus. At the left hand side the edge of a squamous carcinoma of epidermal origin can be seen. H. & E. × 12.

FIG. 7.—Sebaceous carcinoma " ex cyst." Active malignant-looking proliferation of the epithelial lining of a large cyst, presumably originally a " sebaceous " cyst of the kind seen in Fig. 5. The epithelium is in general squamous in type, but large numbers of sebum cells are differentiating out of it. (The sebum cells are seen as pale dots at this power. They are quite typical at higher powers, and their lipid content was confirmed in frozen sections.) H. & E. × 32.



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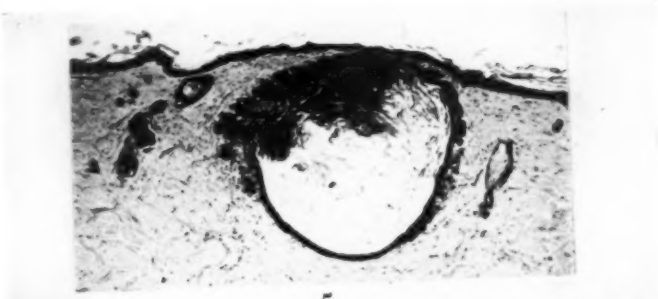

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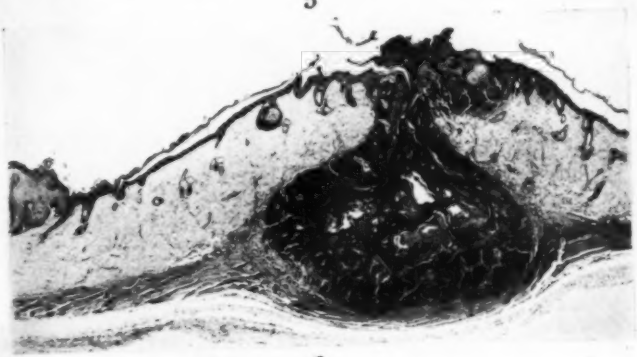

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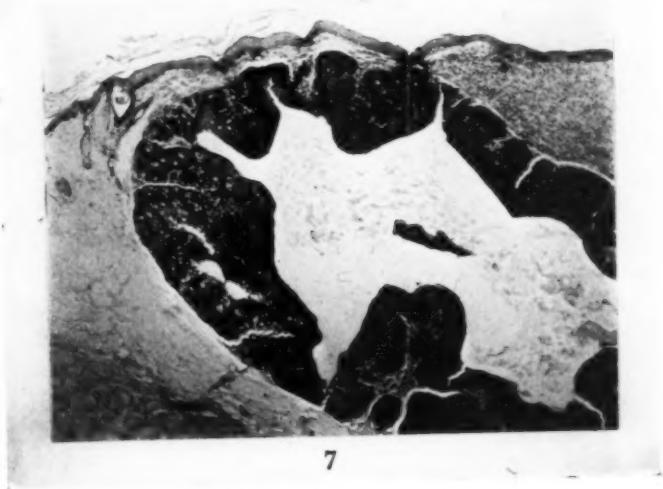

7

CAYLOR, H. D.—(1925) *Ann. Surg.*, **82**, 164.
COTCHIN, E.—(1954) *Vet. Rec.*, **66**, 879.
HEAD, K. W.—(1953) *Ibid.*, **65**, 926.
KING, L. S.—(1947) *Amer. J. Path.*, **23**, 29.
LENNOX, B.—(1954) *Trans. St. John's Hosp. derm. Soc., Lond.*, **33**, 30.
*Idem* AND WELLS, A. L.—(1951) *Brit. J. Cancer*, **5**, 195.
MONTAGNA, W. AND CHASE, H. B.—(1950) *Anat. Rec.*, **107**, 83.
PACK, G. T. AND TABAH, E. J.—(1951) *Arch. Surg.*, **62**, 391.
RUGGLI, P. AND HENZI, E.—(1930) *Helv. chim. Acta.*, **13**, 429.
SALAMAN, M. H.—(1952) *Brit. J. Cancer*, **6**, 155.
WARREN, S. AND WARVI, W. N.—(1953) *Amer. J. Path.*, **19**, 441.
WILLIS, R. A.—(1953) 'Pathology of Tumours.' 2nd Ed. London (Butterworth).

# EXPERIMENTAL PRODUCTION OF INTERSTITIAL CELL TUMOURS.

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THE experimental production of interstitial cell tumours of the testes has been approached from different angles with varying success. Turner (1938) using intra-ocular testicular transplants in castrate rats, demonstrated hypertrophy and hyperplasia of the interstitial cells. Hooker, Gardner and Pfeiffer (1940) produced a large malignant interstitial cell tumour in an A strain mouse by daily oestradiol benzoate injections for 261 days. Changes varying from interstitial cell hyperplasia to benign and histologically malignant interstitial cell tumours were obtained by Skimkin, Grady and Andervont (1941), who implanted stilboesterol-cholesterol pellets into C strain mice ; by Bonsor (1942) and Gardner (1948) using estrogen in A strain mice ; and by Hooker and Pfeiffer (1942) using weekly injections of oestradiol benzoate in A strain mice.

The approach was varied by Biskind and Biskind (1945), who transplanted testes from day-old rats to the spleens of adult male castrate rats. The principles on which this method is based are as follows :

(*a*) By castration, the normal pituitary-testis balance is broken, thus initiating excess secretion of Interstitial Cell Stimulating Hormone by the pituitary.

(*b*) Testosterone produced by the intra-splenic testicular transplant is destroyed in the liver ; and, therefore, the production of I.C.S.H. is not inhibited. Hence the transplant is under continuous excessive pituitary stimulation.

This resulted in one tumour resembling a granulosa cell tumour of the ovary after 11 months. Li, Pfeiffer and Gardner (1947) with a similar technique in mice found no tumours after 275 days. Twombly, Meisel and Stout (1949) are the only workers in this field to show a high rate of tumour formation. Using the above method, they transplanted one-day-old rat testes into the spleens of 60-day-old castrate rats. In 100 animals they had 29 tumours and 28 specimens showed marked interstitial cell hyperplasia. No experiment has been recorded, so far, where an attempt was made to follow the changes taking place in the formation of these tumours. The present work was started in an attempt to learn more about the actual tumour formation.

A review of the relevant litrature indicated that the method just described would be the most suitable and most promising.

However, it also appeared relevant that unpredictable and unexplained reactions to hormone administration in immature animals had been described by Deansley (1928), Butenandt and Kudzus (1935), Rubenstein, Abarbanel and Noder (1938), Nathanson, Franseen and Sweeney (1938) and Warren (1938).

The day-old rat is among the most immature animal at birth and its testis does not enter the scrotum till approximately the 35th day of extra uterine life (Farris and Griffiths, 1942 ; and Wright, 1952). Previous workers had used 60-day-old rats as recipients, but at this age the animal is approaching maturity, when the pituitary is more active, so that the effects of castration would be even greater than in an adult rat. For these reasons it was considered essential to use adult material, in the form of homotransplants, to remove these possible sources of error.

## METHODS AND MATERIALS.

Male Wistar rats of 120 days were anaesthetised and castrated. Part of one testis was transplanted to a subcapsular position in the spleen before castration was completed. The transplant was made, using a wide bore needle in a modification of the punch biopsy technique (Silverman, 1954). Both the above procedures were carried out through one abdominal incision.

At 15-day intervals after operation the animals were killed in batches of 6. A post-mortem examination was made in each case. The transplant-bearing part of the spleen was fixed in Bouins' solution, processed and stained routinely by the Haematoxylin-Eosin method. Sections from most of the tumours were stained by the Masson and Foote techniques to demonstrate the stroma. Some large tumours were divided, a part was reserved in 10 per cent formol-saline for fat staining with Fettrot 7B, and in some cases sections were stained by the Periodic-Acid Schiff technique.

## RESULTS.

In animals killed from 15 days onwards after operation the transplants showed necrotic tubules surrounded by an acute inflammatory reaction, which subsided by the 45th day, leaving the tubules surrounded by fibrous tissue, in which islands of small inactive interstitial cells were visible. In transplants from 45 days to 100 days old, these interstitial cells were seen to enlarge, the cytoplasm became pale, eosinophilic and foamy, and in most, clumps of cells in mitotic division were noted. By 90 days the tubules had disappeared and the islands of interstitial cells were increasing in size, partly due to the individual cells being larger, but more from an increase in the number of cells in the islands; a greater number of cells in mitosis was evident.

The earliest macroscopic tumour was found at 175 days after implantation (Fig. 1). This showed a white elevated mass 4 mm. $\times$ 8 mm., and 3 smaller nodules also at the site of the transplant. These were clearly demarcated from splenic tissue (Fig. 3).

A careful post-mortem examination was carried out on this and all succeeding animals to look for metastases, but no secondary deposits were found. Histological examination showed the mass to be composed of sheets of large polyhedral cells, arranged in clumps of three to twelve cells (Fig. 3 and 4). The cells varied slightly in size and staining properties, some being large, with faintly eosinophilic foamy cytoplasm, while others were smaller, with more densely staining cytoplasm. The eccentric nucleus in both types of cell was vesicular, with peripheral aggregates of chromatin. Mitoses were present but not plentiful. The cellular pattern was supported by a stroma of fine branching fibrous tissue, which emphasised

this distribution of the cells in clumps. The branching stroma was clearly demonstrated by the Masson and Foote techniques (Fig. 5). Numerous sections from this tumour were examined but none showed any histological evidence of malignant change. The cells were strongly sudanophilic, but only faintly stained by the Periodic-Acid Schiff technique.

The experiment covered a period of 415 days after transplantation and during this time 41 tumours were demonstrated in 137 animals, an incidence of 29·9 per cent. Nine animals showed definite hyperplasia of the interstitial cells at the site of transplant. These tumours varied in size from 3 mm. in diameter to 16 × 10 mm. They were usually whitish in colour, and elevated, distending the splenic capsule. Many animals showed small multiple tumours over the site of transplant. Eighteen rats with failed transplants and 24 with vascular adhesions were not counted, as neither of these groups could be expected to produce a tumour. Eight tumour-bearing animals have been preserved alive, following laparotomy, for further transplant experiments. Apart from the last-mentioned group of eight animals, all the other tumours were carefully examined, but none showed any evidence of malignancy. Many of them, however, showed acinar structures (Fig. 6), the cells of which appeared practically identical with those of the surrounding tumour tissue and also gave similar staining reactions with the Haematoxylin-Eosin, Fettrot 7B and Periodic-Acid Schiff techniques.

A control series of 22 animals, prepared by transplanting part of one testis into the spleen as described before, but without castration, showed positive transplants, but no evidence of interstitial cell hyperplasia or tumour formation.

A routine check was made on the pituitary at each post mortem and the general appearance noted. Each pituitary was also weighed and examined histologically. There were no unusual microscopic findings, and the castrate changes were well marked in all cases except the control group. The increase in pituitary weight of a random sample, illustrated in tabular form (Table I), was statistically significant $P < 0{\cdot}001$.

## DISCUSSION.

Interstitial cell tumours are rare in the human, but when they occur in children before puberty they cause the alarming condition of sexual precocity. Bishop (1954) from a review of 17 cases of this condition found the age of onset to lie

## EXPLANATION OF PLATES

FIG. 1.—Spleen from a rat killed 175 days after transplant. Note tumours are elevated from spleen.

FIG. 2.—Spleen from a rat killed 250 days after transplant. Tumour is large and pedunculated. Again smaller tumours are evident.

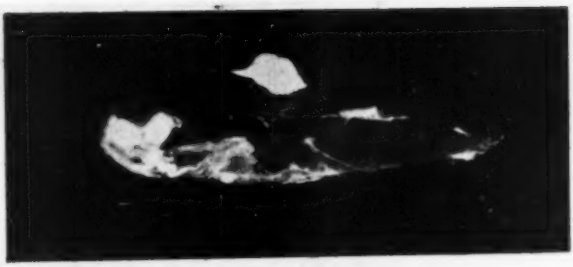
FIG. 3.—A microscopic picture of Fig. 1. Note clear demarcation from splenic tissue. H. & E. × 90.

FIG. 4.—Showing clumping of cells, with fine branching stroma. H. & E. × 100.

FIG. 5.—Stained by the Masson Technique. This demonstrates the branching stroma clearly. × 100.

FIG. 6.—The clock-faced nuclei, with some mitoses, and the foamy nature of the cellular cytoplasm is demonstrated. Cells forming an acinar structure are prominent. H. & E. × 350.

FIG. 7.—Stained by Fettrot 7B. The highly sudanophilic nature of these tumours is evident. × 100.



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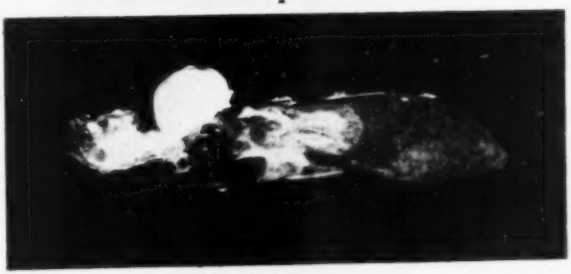

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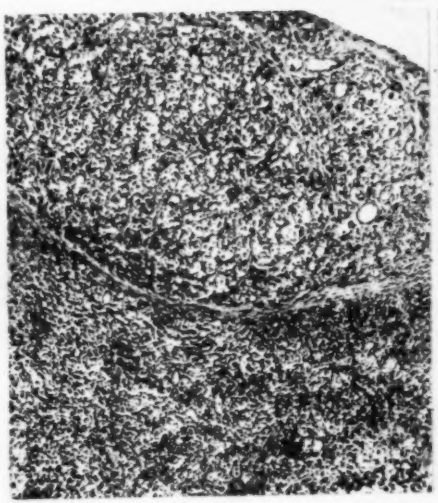

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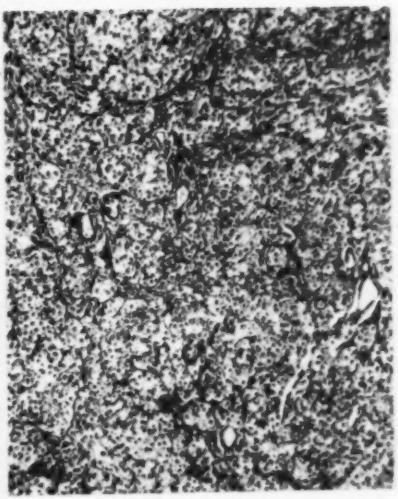

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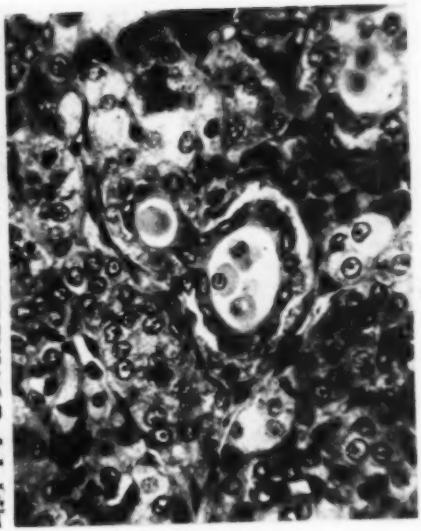

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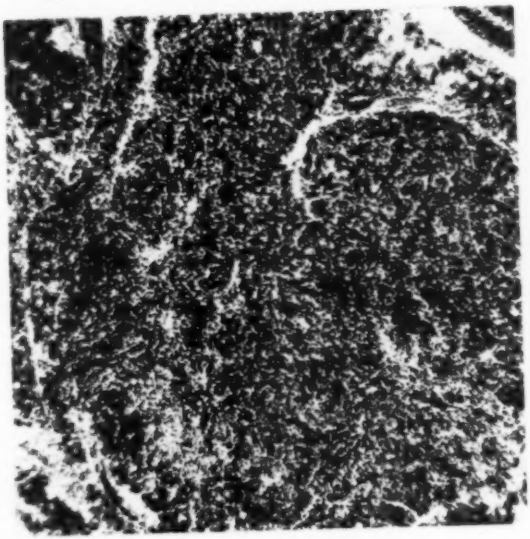

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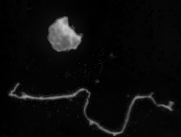

TABLE I.

| Pituitary weight mg. | | Pituitary weight per cent mg. | |
|---|---|---|---|
| Control. | Castrate. | Control. | Castrate. |
| 12·5 | 19·0 | 4·2 | 5·4 |
| 13·5 | 19·0 | 4·1 | 5·1 |
| 12·5 | 19·5 | 3·9 | 5·6 |
| 14·0 | 20·0 | 3·7 | 5·7 |
| 13·0 | 21·0 | 3·6 | 5·7 |
| 13·0 | 20·0 | 4·0 | 5·4 |
| 13·5 | 17·0 | 3·6 | 5·7 |
| 13·0 | 17·5 | 3·9 | 5·5 |
| 13·5 | 17·5 | 3·8 | 5·8 |
| 13·0 | 17·0 | 4·0 | 5·7 |
| 12·0 | 17·5 | 4·0 | 5·4 |
| 13·0 | 16·0 | 3·7 | 5·8 |
| 14·0 | 18·0 | 3·7 | 5·3 |
| 12·5 | 17·0 | 4·2 | 5·2 |
| 13·5 | 19·0 | 3·8 | 5·8 |
| 13·0 | 16·0 | 3·9 | 5·3 |
| 13·0 | 18·0 | 3·5 | 5·1 |
| 12·0 | 18·5 | 4·0 | 5·3 |
| 13·0 | 19·0 | 4·0 | 5·6 |
| 12·0 | 17·0 | 4·0 | 5·7 |
| 12·0 | 17·5 | 4·0 | 5·6 |
| 13·0 | 19·5 | 3·8 | 5·9 |

*Statistical Analysis of Total Pituitary Weights.*

(mg./100 g. rat.)

| Difference of means. | S.E. of difference. | $t$ | Degrees of freedom. | $P$ |
|---|---|---|---|---|
| Castrates − Controls = 1·6910 | 0·1072 | 15·7743 | 124 | <0·001 |

between four and six years in all but three of these. The enlarged testis was not noticed in any case till a cause for the onset of sexual precocity was being sought. Removal of the tumour caused some remission of symptoms in most, but not all these cases. Adult cases are equally rare, but they do not appear to give any endocrinological disturbance. Flynn and Severance (1951), in a review of the literature, quote 20 examples, and add a case of bilateral interstitial cell tumour in a man of 26. In a review of 1032 testicular tumours only 12 of the interstitial cell type were found by Dixon and Moore (1953). Willis (1953) had one example in 50 testicular tumours. All these authors emphasise the difficulty in differentiating between the interstitial cell and the even rarer adrenal rest tumour, only two of which are described by Dixon and Moore (1953). In a series of 85 testicular tumours reported from this department (Jones, 1955) one of each variety was found.

The experimental methods of producing interstitial cell tumours have already been described. The technique adopted in this experiment was in general similar to that of Twombly, Meisel and Stout (1949). It was believed that the results of these authors could have been affected by their using 60-day-old castrate recipients and day-old testis transplants. For this reason homotransplants in 120-day-old castrate rats were used. The results obtained, however, are similar to those of the last mentioned authors. The concept of the part played by

excessive pituitary activity in carcinogenesis, especially in tissues sensitive to hormonal stimulation, has, in recent years, gained popularity to such an extent that hypophysectomy is being carried out as a therapeutic measure in advanced cases of prostatic and breast cancer (Decount, Michard, Weils and Baulieu, 1954; Forrest and Peebles-Brown, 1955).

In this experiment castration caused excessive pituitary gonadotropin secretion, which is reflected in a statistically significant increase in pituitary weights and is believed to have stimulated the interstitial cells to hyperplasia and tumour formation. The findings here would further indicate that the pituitary plays a role in tumorigenesis.

SUMMARY.

1. In a series of 137 castrate rats, with intrasplenic testicular homotransplants, a total of 41 interstitial cell tumours was found, i.e. an incidence of 28·9 per cent. These varied in size from 3 mm. in diameter to 16 × 10 mm.

2. Some animals showed multiple small tumours, while others had solitary nodules.

3. All these tumours were pure interstitial cell growths, they were encapsulated, clearly defined from splenic tissue, and none showed any evidence of malignancy.

4. No tumour formation was found in 22 control animals.

5. The results quoted support the present concept of excessive pituitary stimulation causing tumours of hormonally controlled tissues.

My sincere thanks are due to Professor J. H. Biggart for suggesting this work and for constant criticism and advice. The work was carried out in the Institute of Pathology, The Queen's University of Belfast, while the author was working on a Musgrave Research Studentship.

Mr. D. McA. Mehaffey was responsible for the photography.

## REFERENCES.

Bishop, P.—(1954) 'Recent Advances in Endocrinology.' London (Churchill).

Biskind, M. S. and Biskind, G. R.—(1945) *Proc. Soc. exp. Biol., N.Y.*, **39**, 385.

Bonser, G. M.—(1942) *J. Path. Bact.*, **54**, 149.

Butenandt, A. and Kudzus, H.—(1935) Quoted by Robson, J. M., *Proc. Soc. exp. Biol., N.Y.*, **35**, 49.

Deansly, R.—(1928) *Proc. Roy. Soc.*, **103**, 523.

Decourt, J., Michard, J. P., Weil, B. and Baulieu, E.—(1954) *Bull. Soc. Méd., Paris*, **70**, 699.

Dixon, F. J. and Moore, R. A.—(1953) *Cancer*, **6**, 427.

Farris, E. S. and Griffiths, J. Q.—(1942) 'The Rat.' Philadelphia (Lippincott).

Flynn, P. T. and Severance, A. D.—(1951) *Cancer*, **4**, 817.

Forrest, A. P. M. and Peebles-Brown, D. A.—(1955) *Lancet*, i, 1,054.

Gardner, W. U.—(1948) *Cancer Res.*, **8**, 397.

Hooker, C. W., Gardner, W. U. and Pfeiffer, C. A.—(1940) *J. Amer. med. Ass.*, **115**, 443.

*Idem* and Pfeiffer, C. A.—(1942) *Cancer Res.*, **2**, 759.

Jones, A.—(1955) *Ulster med. J.*, **24**, 27.

Li, M. H., Pfeiffer, C. A. and Gardner, W. U.—(1947) *Proc. Soc. exp. Biol., N.Y.*, **64**, 319.

NATHANSON, I. T., FRANSEEN, C. C. AND SWEENEY, A. R.—(1938) *Ibid.*, **39**, 385.
RUBENSTEIN, H. S., ABARBANEL, A. K. AND NODER, D. V.—(1938) *Ibid.*, **39**, 20.
SILVERMAN, I.—(1954) *J. Amer. med. Ass.*, **155–2**, 1,060.
SKIMKIN, M. B., GRADY, H. G. AND ANDERVONT, H. G.—(1941) *J. nat. Cancer Inst.*, **2**, 65.
TURNER, O. C. D.—(1938) *Amer. J. Anat.*, **63**, 101.
TWOMBLY, G. H., MEISEL, D. AND STOUT, A. P.—(1949) *Cancer*, **2**, 884.
WARREN, S.—(1938) Quoted by Nathanson *et al.* *Proc. Soc. exp. Biol., N.Y.*, **39**, 385.
WILLIS, R. A.—(1953) 'Pathology of Tumours.' London (Butterworth).
WRIGHT, S.—(1952) 'Physiology.' London (Oxford University Press).

# IMMUNITY TO TRANSPLANTED TUMOUR: THE EFFECT OF TUMOUR EXTRACTS ON THE GROWTH OF HOMOLOGOUS TUMOURS IN RATS.

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IMMUNITY to transplanted tumours in animals has been extensively investigated although failure to control genetic factors rendered much of the earlier work of doubtful value. Following the criticisms of Woglom (1929), greater attention was paid to experimental design particularly with regard to the host-tumour relationship and in a number of studies, immunity has been produced against tumours which arose in, and grew progressively in inbred strains of animals (Hauschka, 1952 ; Stern, 1953). In a series of studies reported by Aptekman, Lewis and King (1946, 1949) and by Lewis, King, Aptekman and Seibert (1948), immunity to transplanted tumours in inbred rats was induced following destruction of growing tumours by injecting them with ethanol extracts of homologous tumour tissue and by pre-treating tumour-susceptible rats with the tumour extracts. The tumours used in these studies were induced in inbred strains of rats and were transplanted into animals of the same strains. In most cases, however, the tumours had been repeatedly sub-passaged and it was suggested by Hauschka (1952) that this repeated transplantation may have produced immunogenetic differences between tumour and host strain of animals which would favour a refractory state following tumour destruction.

In the following experiments, the effect of tumour extracts on tumour growth has been studied using methylcholanthrene-induced tumours in inbred rats. Initially, these tumours had not been through many transplant generations so that any effect due to repeated tumour transplantation would be minimized. Experiments were also performed using tumours which had been sub-passaged through known numbers of generations so that any effect due to repeated tumour transplantation could be determined.

## MATERIALS AND METHODS.

### *Animals and tumours.*

Experiments were performed with inbred rats of a Wistar strain. A number of litters of this inbred strain were obtained from Boots Pure Drug Co., Ltd., Nottingham, and tumours were induced in one rat of each litter following a single subcutaneous injection of methylcholanthrene. Each tumour grew progressively on implantation into the offspring produced by mating the litter mates of the tumour donor rat although the growth rate of the different tumours varied considerably. The tumours were designated as follows :

*Tumour S*69 (Group I).—A highly cellular anaplastic sarcoma which grew rapidly so that host rats had to be killed within 10 to 14 days.

*Tumour S5* (Group B).—A moderately cellular fibrosarcoma which grew fairly rapidly, reaching palpable size in 4 to 5 days. Rats bearing this tumour had to be killed within 3 to 4 weeks.

*Tumour S66* (Group III).—A moderately cellular sarcoma showing some differentiation into fibrous tissue. Implants of this tumour reached palpable size within 9 to 12 days and grew fairly slowly so that host rats had to be killed within 5 to 7 weeks.

Tumour implants were made subcutaneously in the right dorsal region using a No. 15 gauge trocar and cannula. The tumour tissue used at each transplant was obtained under sterile conditions from the healthy peripheral part of a single tumour, and was sectioned into small pieces just large enough to fit into the trocar. The tumour graft in all cases consisted of approximately one half trocar full of tumour tissue.

*Preparation of tumour extracts.*

Rats bearing tumours of a suitable size were killed by fracture of the spinal cord and the tumours were quickly removed to ice-cooled containers. The tumour tissue was pooled after the removal of any necrotic tissue and homogenised in an ice-cooled, vertical, top drive, homogeniser. The tumour homogenate was mixed with an equal amount of 95 per cent ethanol (1 ml. per gram of tumour), allowed to stand at 4° C. for 24 hours and then treated with a further two volumes of ethanol. This facilitated the separation of the tumour residue which was removed by centrifugation (3000 r.p.m. and 0° C.). The final tumour extracts were pale yellow in colour with an ethanol content of 75 to 80 per cent. These extracts were concentrated at 30–34° C. under reduced pressure to one-tenth of their original volume.

The concentrated ethanol extracts contained considerable amounts of white insoluble material. No attempt was made to separate this insoluble material or to adjust the ethanol concentration of the tumour extracts used in the treatment of tumours. Dry weight determinations at 110° C. indicated that the extracts contained amounts of total solids varying between 40 and 70 milligrams per millilitre.

*Experimental procedure.*

Experiments were performed using whole litters of rats, and in each case a number of implanted rats were left untreated to serve as controls. Rats bearing tumour implants which had grown from 4 to 12 days (depending upon the tumour used) and had reached a size of approximately 20 × 10 × 10 mm. were treated with the appropriate tumour extract concentrate. Injections were made directly into the tumours, each rat receiving 0·5 or 1·0 ml. of the extract daily until the tumour regressed or until it became evident that tumour growth would continue and the rats were killed. Rats in which tumours had regressed following treatment were tested for immunity 3 to 4 weeks after the original implantation site had healed. Challenge grafts which were made in the left flank were approximately one-half the size of the original graft. Rats in which the challenge graft failed to grow were re-implanted one month later with large amounts of tumour (two or three trocars full) and kept under observation for at least six months.

Attempts were also made to induce tumour immunity by pre-treating rats with the tumour extract concentrates. In these experiments, rats of several litters

were injected subcutaneously with the appropriate tumour extract. Each rat received a total of 4·5 ml. of tumour extract concentrate in doses of 0·5 ml. over a period of three weeks. Three weeks after the last injection, the treated rats together with a number of untreated litter mates as controls were implanted with challenge grafts of the appropriate tumour.

## RESULTS.

The results of treating growing tumours by injecting them with ethanol extract concentrates of homologous tumour tissue are shown in Table I. Considerable damage was caused in tumours treated with the tumour extracts and they became blackened and dry. This damage was often brought about by as few as 3 to 5 injections of the tumour extracts and in a number of experiments the damaged tumours regressed, leaving a well-healed scar at the site of implantation. Usually, however, treated tumours continued to grow, even though treatment with the extracts was continued, until eventually the rats had to be killed. Altogether 104 rats bearing implants of 3 different tumours were treated with tumour extract concentrates prepared from 13 different collections of tumour tissue, and out of these only 5 tumours regressed completely. In a number of experiments further tumour growth was observed 2 to 4 weeks after the original tumour implant had

TABLE I.—*Results of Treating Transplanted Tumours with Homologous Tumour Extract Concentrates.*

| Tumour and litter of origin. | Transplant generation treated. | Tumour extract concentrate injected.* | Total volume of concentrate injected (ml.). | Number of animals treated. | Number of tumours regressing. | Number of untreated animals inoculated.† |
|---|---|---|---|---|---|---|
| S5 (Group B) | 8 | 6 S5 | 20 | 2 | 0 | 3 |
| | 9 | 8 S5 | 11 | 10 | 0 | 5 |
| | 11 | 9 S5 | 20 | 11 | 0 | 5 |
| | 14 | 11 S5 | 26 | 12 | 1 | 4 |
| | 19 | 13 S5 | 19 | 8 | 0 | 6 |
| | 20 | 18 S5 | 14 | 5 | 0 | 8 |
| Total | — | — | — | 48 | 1 | 31 |
| S69 (Group I) | 3 | 1 S69 | 12 | 6 | 0 | 3 |
| | 4 | 1 S69 | 16 | 3 | 0 | 3 |
| | 15 | 2 S69 | 23 | 5 | 1 | 3 |
| | 16 | 5 S69 | 14 | 12 | 0 | 4 |
| Total | — | — | — | 26 | 1 | 13 |
| S66 (Group III) | 3 | 1 S66 | 46 | 7 | 0 | 5 |
| | 5 | 3 S66 | 30 | 6 | 0 | 3 |
| | 6 | 4 S66 | 20 | 6 | 0 | 3 |
| | 9 | 7 S66 | 18 | 11 | 3 | 6 |
| Total | — | — | — | 30 | 3 | 17 |
| Total | — | — | — | 104 | 5 | 61 |

* Figures indicate the transplant generation of tumour used for the preparation of the tumour extracts.

† Tumour grew in all untreated animals.

regressed following treatment. None of the recurrent tumour growths regressed following further treatment with tumour extract. The 5 rats in which tumours had regressed completely were re-implanted several times over a six-month period with large amounts of the appropriate tumour and all remained immune to tumour growth.

The experiments summarised in Table II show that pre-treatment of tumour-susceptible rats with tumour extract concentrate was ineffective in producing tumour immunity. Thus none of the 47 rats pre-treated with the appropriate tumour extract proved to be immune upon implantation with the homologous tumour and the tumour grafts grew as rapidly as the implants in untreated control animals.

TABLE II.—*The Effect of Prior Injections of Tumour Extract Concentrates on the Growth of Implants of the Homologous Tumours.*

| Tumour and litter of origin. | Tumour extract concentrate injected.* | Number of rats treated. | Number resistant to growth of tumour implants. | Number of untreated animals inoculated.† |
|---|---|---|---|---|
| S5 (Group B) | 19 S5 | 8 | 0 | 6 |
| | 26 S5 | 12 | 0 | 3 |
| S66 (Group III) | 8 S66 | 12 | 0 | 3 |
| | 12 S66 | 15 | 0 | 3 |
| Total | — | 47 | 0 | 15 |

* Figures indicate the transplant generation of tumour used for the preparation of the tumour extracts.

† Tumour grew in all untreated animals.

## DISCUSSION.

In most cases where immunity has been induced against tumours which originated in inbred strains of animals, the tumours had been repeatedly sub-passaged over long periods of time. It is now generally agreed that mutation of tumour may occur during such procedures resulting in the development of immunogenetic differences between tumour and host which facilitate the development of tumour immunity. The tumours used in the present experiments were induced in highly inbred rats and had been sub-passaged only a few times, so that the chance of tumour mutation was minimised. Attempts to induce regression of these tumours by treating them with ethanol extracts of homologous tumour tissue were nearly always unsuccessful and no difference was noted in the response to treatment of implants of the different tumours between the 3rd and 20th generation of transfer (Table I). Similarly treatment of rats with the tumour extracts failed to alter their susceptibility to tumour growth and tumour grafts grew in all treated animals.

These results fail to confirm the findings of Aptekman, Lewis and King (1946, 1949), although they are in agreement with the results of recent studies, where attempts to repeat earlier work using tumours which arose spontaneously in inbred animals and had been sub-passaged in animals of their strain of origin through only a few transplant generations were completely unsuccessful (Fardon and Prince, 1953; Foley, 1953; Goldfeder, 1954). Thus Goldfeder showed that tumour resistance could be induced in inbred rats (Bagg strain) following regression of X-irradiated grafts of an autogenous tumour. Although this tumour arose in a

rat of the Bagg strain, it had reached its 75th generation of transfer at the time of the experiment. Attempts to repeat this work using three highly inbred strains of mice (C57BL, $C_3H$ and DBA) and two inbred strains of rats (A $\times$ C and August) and with tumours originating in these inbred strains have been unsuccessful (Goldfeder, 1953 ; 1954).

The possibility that the tumour extracts used in the present study were inactive cannot be ruled out, although this is unlikely since the extracts were prepared under conditions similar to those used by Lewis, King, Aptekman and Seibert (1948). In addition, the tumour extract concentrates usually caused considerable damage to tumours, especially in the earlier stages of treatment. In almost every case, however, the damaged tumours continued to grow even though treatment with the tumour extracts was continued.

In the studies of Aptekman, Lewis and King (1946) practically all of the tumours treated with ethanol extracts of homologous tumour tissue regressed leaving the cured rats resistant to further implants of the original tumour. It was also observed, however, that tumour regression and immunity could be induced using ethanol extracts of non-related human tumour tissue. These results suggest that some other factor, probably depending upon antigenic differences between tumour and host strain of animals, is responsible for the induction of tumour immunity following tumour destruction by some " toxic " agent in the tumour extracts. This would explain the failure of the present experiments since the tumours used had been sub-passaged only a few times so that the chance of tumour mutation was minimized. Under these circumstances immunogenetic differences between tumour and host may not have existed, or more likely not have been great enough to elicit an immune response sufficient to permit the development of tumour immunity.

The nature of the tumour destroying substance in the tumour extracts is still unknown, although comparative studies of the chemical composition of ethanol extracts of malignant and normal animal tissue have revealed significant differences in the concentration of many of the known constituents, including carbohydrate and bound and free amino acids. (Seibert, Soto-Figueroa, Miller, Seibert, Aptekman and Lewis, 1954 ; Seibert, Soto-Figueroa, Miller and Seibert, 1954). It was suggested by Hauschka (1952), in reference to the work of Aptekman, Lewis and King (1946), that lipid or lipoprotein antigens may be involved in the production of tumour regression and immunity by means of ethanol extracts of tumour tissue. However, this suggestion could not be substantiated since little or no lipid material was detected in the tumour extracts. Perrault and Shear (1955) have isolated polysaccharide-containing fractions from a number of animal tumours with biological properties similar to those of certain bacterial polysaccharides. These fractions were shown to induce haemorrhagic necrosis in tumours following intraperitoneal injection into tumour--bearing animals and they also inhibited growth of several animal tumours. It is possible that the tumour destroying and toxic activity of ethanol extracts of rat tumour tissue may depend upon the presence of similar polysaccharides.

## SUMMARY.

Rats bearing grafts of tumours originally induced within the same inbred strain of animals were treated with ethanol extract concentrates of the homologous tumours. The tumour extracts caused considerable tissue destruction when

injected into growing tumors, although only a few of the treated tumours regressed completely. Where treated tumours regressed and the hosts remained free from recurrent growth of the original graft, the hosts were found to be immune to further implants of the same tumour. Treatment of rats with the tumour extract concentrates prior to implantation of tumour failed to induce any immunity and the tumour grafts grew as rapidly as those in untreated animals.

It is suggested that the development of tumour immunity depends upon the presence of immunogenetic differences in the tumour-host relationship and that the ethanol extracts of tumour tissue act as tumour destroying agents which inhibit tumour growth and so permit the development of immune processes.

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## REFERENCES.

APTEKMAN, P. M., LEWIS, M. R. AND KING, H. D.—(1946) *J. Immunol.*, **52**, 77.—(1949) *Ibid.*, **63**, 435.
FARDON, J. C. AND PRINCE, J. E.—(1953) *Cancer Res.*, **13**, 9.
FOLEY, E. J.—(1953) *Ibid.*, **13**, 578.
GOLDFEDER, A.—(1953) *J. nat. Cancer Inst.*, **14**, 721.—(1954) *Brit. J. Cancer*, **8**, 320.
HAUSCHKA, T. S.—(1952) *Cancer Res.*, **12**, 615.
LEWIS, M. R., KING, H. D., APTEKMAN, P. M. AND SEIBERT, F. B.—(1948) *J. Immunol.*, **60**, 517.
PERRAULT, A. AND SHEAR, M. J.—(1955) *Proc. Amer. Ass. Cancer Res.*, **2**, 39.
SEIBERT, F. B., SOTO-FIGUEROA, E., MILLER, E. E. AND SEIBERT, M. V.—(1954) *Growth*, **18**, 145.
*Iidem*, Aptekman, P. M. and LEWIS, M. R.—(1954) *Ibid.*, **18**, 1.
STERN, K.—(1953) *Chicago med. Sch. Quart.*, **14**, 68.
WOGLOM, W. H.—(1929) *Cancer Rev.*, **4**, 129.

# IMMUNITY TO METHYLCHOLANTHRENE-INDUCED TUMOURS IN INBRED RATS FOLLOWING ATROPHY AND REGRESSION OF THE IMPLANTED TUMOURS.

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In a series of investigations using tumours which were originally induced with methylcholanthrene in inbred rats, it was shown that atrophy and regression of tumours caused by restricting their blood supply rendered the cured rats immune to subsequent inoculations of tumour, (Lewis, Maxwell and Aptekman, 1951 ; Lewis and Aptekman, 1952). Attempts to confirm these experiments using tumours which arose spontaneously in inbred strains of mice and had been subpassaged only a few times in animals of their strain of origin were unsuccessful (Fardon and Prince, 1953 ; Foley, 1953*a*) and it was suggested that the success of the earlier studies of Lewis and Aptekman (1952) was due to the presence of immunogenetic differences between tumour and host which probably arose during repeated transplantation of tumour. Later, however, Foley (1953*b*) showed that immunity could be induced in $C_3H$ mice following regression of tumours originally induced with methylcholanthrene, and so it would seem that the immune response evoked following tumour atrophy varies with the two types of tumour.

The development of tumour immunity in inbred rats following atrophy and regression of tumours originally induced with methylcholanthrene has been re-investigated and in the following experiments, a number of tumours have been studied at different stages of their transplantation history in an effort to determine the nature of the antigenic stimulus producing this immunity.

## MATERIALS AND METHODS.

The tumours described in a previous paper (Baldwin, 1955), together with three additional tumours, were used in the present experiments. These tumours were induced with methylcholanthrene in rats of selected inbred litters and each tumour was transplanted only into the offspring produced by mating litter mates of the tumour donor rat. The new tumours were designated as follows :

Sarcoma S67 : A very hard tumour which reached palpable size within 12 days of implantation in Group II rats and then grew very slowly until host rats had to be killed within 6 to 10 weeks.

Sarcoma S606 (Group 13 rats) and Sarcoma S609 (Group 12 rats) : Both tumours were moderately active, growing to palpable size within 8 days of implantation. Rats bearing these tumours became moribund within 4 to 6 weeks and were killed.

## EXPERIMENTAL METHODS.

The technique of tumour transplantation has been fully described in a previous paper (Baldwin, 1955). Rats bearing tumour grafts consisting of approximately

one half trocar full of tumour tissue were examined three times a week; tumour sizes being measured in three dimensions. When engrafted tumours reached a size of approximately 20 × 10 × 10 mm. (Table I), they were ligated so as to restrict their blood supply. This was achieved in anaesthetised rats by firmly drawing a loop of suture nylon under the base of the tumour which was lifted free from the muscles on the back of the rat so that the tumour was enclosed within a fold of loose skin. Further loops of nylon were placed around the skin pockets at 24 hour intervals until the tumours regressed. Tumours usually became discoloured and considerably swollen within 24 hours of ligation. Then, following absorption of the tumours, the skin pockets slowly dried up over several days, leaving small scars at the tumour implant sites.

Rats in which ligated tumours had regressed were challenged for immunity 3 to 4 weeks later by re-implantation of the appropriate tumour. Challenge grafts

TABLE I.—*Inhibition of Tumour Growth following Ligation of Transplanted Tumours in Inbred Rats.*

| Tumour and litter of origin. | Transplant generation treated. | Number of days tumour had grown before treatment. | Number of tumour-bearing rats treated. | Number of tumours regressing. |
|---|---|---|---|---|
| S5 (Group B) | 19 | 6 | 5 | 5 |
| | 20 | 5 | 19 | 19 |
| | 22 | 4 | 6 | 6 |
| | | | 30 | 30 |
| S69 (Group I) | 9 | 5 | 10 | 5 |
| | 16 | 3 | 9 | 6 |
| | 19 | 4 | 11 | 11 |
| | 25 | 4 | 11 | 6 |
| | 26 | 4 | 7 | 6 |
| | | | 48 | 34 |
| S67 (Group II) | 4 | 17 | 3 | 3 |
| | 5 | 10 | 9 | 9 |
| | 6 | 10–14 | 17 | 17 |
| | 7 | 8 | 12 | 12 |
| | 8 | 10 | 9 | 9 |
| | | | 50 | 50 |
| S66 (Group III) | 8 | 5 | 3 | 2 |
| | 9 | 10 | 14 | 14 |
| | 10 | 7–10 | 15 | 12 |
| | 12 | 7 | 11 | 10 |
| | | | 43 | 38 |
| S609 (Group 12) | 2 | 6 | 8 | 8 |
| | 3 | 8 | 11 | 11 |
| | 4 | 7–10 | 16 | 15 |
| | 6 | 5 | 4 | 4 |
| | | | 39 | 38 |
| S606 (Group 13) | 2 | 6–8 | 13 | 12 |
| Total | | | 223 | 202 |

were made in the left side of the rat and were approximately half the size of the initial implant. Any rats proving to be resistant to the challenge grafts were re-implanted with large amounts of tumour (1 to 2 trocars full) 1 to 2 months later and kept under observation for at least 6 months. During this time the tumour implantation sites were examined twice weekly, and at the conclusion of each experiment a number of rats were examined for metastatic growth.

## RESULTS.

The results of experiments in which transplanted tumours in inbred rats were ligated in skin pockets so as to occlude their blood supply are recorded in Table I. In most cases it was possible to isolate completely the small progressively growing tumour grafts and so obstruct their blood supply. Occasionally, however, tumour grafts invaded the muscle tissue of the host so that it was not possible to ligate the whole tumour. In these experiments the tumours continued to grow even though the treatment destroyed practically all of the original tumour grafts. This often happened in experiments with the rapidly growing tumour S69 and also occasionally with tumour S66 (Table I).

The results recorded in Table II show that out of 197 rats in which tumours had regressed as a result of ligation only 77 (39 per cent) were resistant to the first challenge graft of the original tumour. However, the immune response evoked following atrophy and regression of the different tumours varied considerably and appeared to be related to the growth rate of the tumours. Thus regresssion of the rapidly growing tumour S69 induced tumour resistance in only 1 cured rat (3 per cent) and regression of two other fast-growing tumours, S5 and S606, produced similar results. In contrast, experiments performed with tumour S67, which had a relatively slow growth rate, were nearly always successful, immunity being produced in 76 per cent of the cured rats. The results obtained with tumours S66 and S609 were more variable, although approximately one half of the cured rats were found to be resistant to tumour growth.

As shown in Table II, repeated transplantation of tumour had no great influence on the ability of atrophying tumour to induce a resistant state. Thus in experiments with tumour S67, it was found that immunity could be induced using tumour grafts from the 4th to the 8th generation of transfer. Similar results were obtained in experiments where regression of tumour induced little or no tumour resistance. For example, regression of grafts of tumour S69 from between the 9th and 26th generation induced a resistant state in only one rat.

Most of the rats in which the first challenge graft had failed to grow were found to be immune to re-implantation with large doses of the appropriate tumour, and out of 77 rats challenged only 3 (4 per cent) proved to be susceptible to tumour growth.

## DISCUSSION.

The results obtained in the above experiments confirm the findings of Lewis and Aptekman (1952) and Foley (1953*b*) regarding the development of immunity in inbred animals to transplanted tumours originally induced with methylcholanthrene. However, the immune response evoked following atrophy and regression of different tumours was found to vary considerably, depending upon their growth rate. Thus regression of rapidly-growing tumours as a result of ligation induced

TABLE II.—*Development of Tumour Immunity in Inbred Rats following Induced Regression of Tumours Originating in the Same Strain.*

| Tumour and litter of origin. | Transplant generation used for first graft. | Number of rats in which tumours had regressed. | Number of cured rats immune upon re-implantation. | Per cent immune. | Transplant generation of tumour used for challenge graft. | Number of untreated rats inoculated.* |
|---|---|---|---|---|---|---|
| S5 | 19 | 5 | 3 | | 19 | 3 |
| (Group B) | 20 | 19 | 2 | | 21 | 8 |
| | 22 | 6 | 0 | | 23 | 8 |
| | | 30 | 5 | 17 | | 19 |
| S69 | 9 | 5 | 0 | | 14 | 10 |
| (Group I) | 16 | 6 | 0 | | 17 | 4 |
| | 19 | 9 | 1 | | 21 | 6 |
| | 25 | 6 | 0 | | 26 | 10 |
| | 26 | 6 | 0 | | 27 | 5 |
| | | 32 | 1 | 3 | | 35 |
| S67 | 4 | 3 | 3 | | 5 | 9 |
| (Group II) | 5 | 9 | 9 | | 5 | 4 |
| | 6 | 16 | 15 | | 6 | 4 |
| | 7 | 12 | 5 | | 7 | 5 |
| | 8 | 9 | 5 | | 8 | 3 |
| | | 49 | 37 | 76 | | 25 |
| S66 | 8 | 2 | 1 | | 8 | 5 |
| (Group III) | 9 | 14 | 8 | | 9 | 9 |
| | 10 | 12 | 0 | | 10 | 4 |
| | 12 | 8 | 8 | | | 10 |
| | | 36 | 17 | 47 | | 28 |
| S609 | 2 | 8 | 8 | | 2 | 4 |
| (Group 12) | 3 | 11 | 6 | | 3 | 9 |
| | 4 | 16 | 1 | | 4 | 3 |
| | 6 | 3 | 2 | | 7 | 3 |
| | | 38 | 17 | 45 | | 19 |
| S606 (Group 13) | 2 | 12 | 0 | 0 | 2 | 2 |
| Total | | 197 | 77 | 39 | | 128 |

* Tumour grew in all untreated animals.

little or no tumour immunity, whereas treatment of tumour S67, which has a slow growth rate, rendered nearly all the cured hosts resistant to further implants of the original tumour. It should be pointed out that the method of testing for tumour immunity by re-implantation of tumour is an all-or-none technique which will not detect slight levels of resistance. In addition, the minimum level of resistance which will protect a cured host against re-implants of tumour will depend in part upon the growth rate or " virulence " of the tumour, and so this may explain why experiments with rapidly-growing tumours were unsuccessful.

The nature of the antigenic stimulus evoking an immune response following regression of transplanted tumour in inbred animals is still unknown, although it

is now generally assumed that immunogenetic differences between tumour and host rather than specific tumour antigens are responsible for the induction of tumour immunity (Hauschka, 1952 ; Eichwald, 1953 ; Cohen and Cohen, 1954). Evidence to support this assumption has been obtained by Foley (1953*a*, 1953*b*), who showed that immunity could be induced in inbred mice against transplanted tumours which were induced originally with methylcholanthrene, but not against tumours which originated spontaneously in the same strain of mice.

According to Hauschka (1952) these immunogenetic differences arise during repeated subpassaging of tumour over long periods of time by mutation of either the tumour or host strain of animals. Although heterogeneity in the host strain of animals cannot be ruled out as a contributory factor, Foley (1953*a*) showed that this factor did not influence the immune response induced in mice following regression of transplanted tumours which arose spontaneously, and no immunity was produced. Repeated subpassaging of tumour also appears to have no great influence on the ability of atrophying tumour to induce tumour resistance, and in the present experiments, as well as those of Foley (1953*b*), it was possible to induce immunity before repeated tumour transplants had been made. Similarly, in the present study, attempts to induce immunity against a rapidly-growing tumour, S69, were nearly always unsuccessful even though experiments were performed with tumour grafts from between the 9th and 26th generation of transfer.

Thus if the induction of tumour immunity depends upon immunogenetic differences between tumour and host, then it would seem that the original induced tumour represents a mutated tissue capable of eliciting an immune response when implanted into the host strain of animals. This suggestion will require further study however, especially in view of Burdette's recent criticism of the correlation between mutagenicity and carcinogenicity of chemical compounds (Burdette, 1955).

A number of techniques have been described recently by which the antibody response to tumour inoculation can be determined. These include agglutination reactions using erythrocytes (Gorer and Mikulska, 1954) and leucocytes (Amos, 1953) and the anaphylaxis technique which was used by Fink, Snell and Kelton (1953) to determine the antibody response of Balb. C mice following inoculation of an homologous tumour. It may well be that the application of these techniques to the study of the antigenic structure of transplanted tumours which originated spontaneously or were induced with methylcholanthrene in inbred animals will disclose the nature of the antigenic stimulus producing immunity following induced regression of the latter type of tumour.

## SUMMARY.

Immunity to transplanted tumours originally induced with methylcholanthrene has been produced in inbred rats following atrophy and regression of tumour grafts ligated so as to restrict their blood supply.

The immune response evoked following regression of tumour was found to vary considerably with different tumours. In some cases regression of tumour induced immunity in practically all cured rats, whereas with other tumours none of the cured rats were resistant to re-implants of the original tumour. Repeated subpassaging of tumour did not influence the ability of atrophying tumour to induce tumour resistance, and in cases where it was possible to induce immunity experiments

performed with tumours which had been transplanted only a few times were successful.

It is suggested that the antigenic stimulus producing immunity to transplanted tumours which were originally induced in inbred rats with methylcholanthrene depends upon immunogenetic differences between tumour and host, and the origin of these differences is discussed.

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## REFERENCES.

Amos, D. B.—(1953) *Brit. J. exp. Path.*, **34**, 464.
Baldwin, R. W.—(1955) *Brit. J. Cancer*, **9**, 646.
Burdette, W. J.—(1955) *Cancer Res.*, **15**, 201.
Cohen, A. and Cohen, L.—(1954) *Brit. J. Cancer*, **8**, 313.
Eichwald, E. J.—(1953) *J. nat. Cancer Inst.*, **14**, 705.
Fardon, J. C. and Prince, J. E.—(1953) *Cancer Res.*, **13**, 9.
Fink, M. A., Snell, G. D. and Kelton, D.—(1953) *Ibid.*, **13**, 666.
Foley, E. J.—(1953*a*) *Ibid.*, **13**, 578.—(1953*b*) *Ibid.*, **13**, 835.
Gorer, P. A. and Mikulska, Z. B.—(1954) *Ibid.*, **14**, 651.
Hauschka, T. S.—(1952) *Ibid.*, **12**, 615.
Lewis, M. R. and Aptekman, P. M.—(1952) *Cancer*, **5**, 411.
*Idem*, Maxwell, D. B. and Aptekman, P. M.—(1951) *Surgery*, **30**, 689.

# ON THE CATALASE ACTIVITY OF THE LIVER SOLUBLE PROTEINS IN RATS FED ON 4-DIMETHYLAMINOAZOBENZENE AND LOCALIZATION OF THE PROTEIN-BOUND DYES.

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AMONG all the tissues of the tumour-bearing animal the liver appears to be the most sensitive to the presence of the tumour. Properly it is in the field of enzymology that this sensitivity attains its clearest expression by the lowering of the liver catalase activity. This interesting phenomenon arises systematically with all spontaneous, induced or transplantable tumours in rats and mice of various strains (Colwell, 1910; Rosenthal, 1912; Magat, 1924; Greenstein, 1943, 1954) and also in frogs (Lucké and Berwick, 1954) and chickens (Adams, 1954). Hepatomas, lymphomas, sarcomas, carcinomas, etc., all exert a similar depressing effect on the liver catalase activity as if the tumours, regardless of etiology or histogenesis, converge towards a common type of tissue possessing an identical property.

Now if we consider that of all the individual enzyme systems examined in the tumour-bearing host, only the hepatic catalase is firmly and markedly affected, and that in other conditions, in pregnancy and after subcutaneous embryonic implant, there is no change in the normal level of the liver catalase, we can reach the suggestive conclusion that the depression of the catalase is an exclusive expression of the cancerous affection. This conclusion is also in accordance with the reversibility of the enzymatic activity after the complete removal of the tumour.

But the possibility of applying this finding to early diagnosis of human cancer has been unsuccessful, and Young, Collier and Homburger (1947), and Mutolo and Abrignani (1954) found no difference in catalase activity between men suffering from cancer and those in normal health.

To explain the decrease of the catalase activity Weil-Malherbe and Schade (1948) hypothesized that this phenomenon might be caused by the reduction of the catalase synthesis following the high protein requirements of the tumour; however the liver catalase of the tumour-bearing animals, regardless of the nature of the diet, was constantly lowered.

The eventuality of the humoral transmission of some anticatalase agent from the tumour has been examined by Lucké, Berwick and Zecker (1952) who showed that in parabiotic rats the level of the liver catalase activity of the non-tumour-bearing partner is reduced. Furthermore Nakahara and Fukuoka (1950) succeeded in isolating from a number of human tumours a protein-like toxic substance (toxohormone) which, when injected into mice, brings about a lowering in the liver catalase activity. Finally Hargreaves and Deutsch (1952) found that boiled extracts of tumours could inhibit *in vitro* the activity of crystalline catalase.

Starting with this observation we have discovered that the ovomucoid acquires, after boiling, a strong inhibitory power against catalase and we have supposed that

probably the anticatalase activity of the tumours may be due to a glycoprotein (Abrignani and Mutolo, 1954).

The mechanism of the fall in the liver catalase activity in tumour-bearing animals and why this effect begins to be readily measurable only at a stage when the tumour is at least 5 per cent of the body weight of the host is not yet understood. It is possible that the toxic substance elaborated in relatively insufficient amounts by a small size tumour does not affect the liver catalase since probably it is neutralized in the blood stream before entering the liver. Hence the lowering of the hepatic catalase activity should be observable not only when the small tumour itself grows in the liver but also in the earliest steps of the neoplastic induction.

In an attempt to prove the above-mentioned hypothesis we have studied the behaviour of the catalase activity during the first steps of the liver carcinogenesis in rats fed on 4-dimethylaminoazobenzene (DAB).

In regard to this Miller and Miller (1947, 1953), have demonstrated that protein-bound dyes appear in the liver shortly after the feeding of DAB and continue to accumulate until a maximum level is reached at about one month ; thereafter the level decreases progressively (Miller and Miller, 1952). In contrast, these protein-bound dyes are absent in the fully developed liver tumours (Miller and Miller, 1947 ; Price, Miller, Miller and Weber, 1949).

The studies on the metabolism of this dye have shown that the major share of of the dye is metabolized via reductive cleavage of the azo-linkage, hydroxylation of the ring in the 4′-position, and oxidative removal of the N-methyl groups. Only a small percentage of a dye derivative becomes chemically bound to certain of the liver proteins (McDonald, Plescia, Miller and Miller, 1953 ; Stevenson, Dobriner and Rhoads, 1942). These protein-bound dyes are highly specific for the induction of the hepatoma in the rat liver, since they cannot be detected in the liver of the animals which are not susceptible to the carcinogenic action of DAB (Miller and Miller, 1952).

The riboflavin, added to the diet, was discovered to be one of the most potent inhibitors of the induction of the tumours by DAB (Kensler, Sugiura, Young, Halter and Rhoads, 1941).

Furthermore it has been observed that the major portion of the protein-bound amino azo dye derivatives is contained in the slower sedimenting class of soluble, non-particulate proteins of rat liver (Sorof, Cohen, Miller and Miller, 1951) and on electrophoretic analysis the protein-bound dyes were found approximately to relate to the slowly moving *h* component (Sorof, Golder and Ott, 1954).

The present paper deals with the catalase activity of the liver soluble, non-particulate, proteins of rats feeding for a week on DAB. For the purpose of this research the isolation and the separation of the liver soluble proteins have been performed by salting-out method which has allowed us to localize both the fraction containing the protein-bound dyes and that possessing the catalase activity.

## METHODS.

*Care of animals.*—In all, 40 albino rats young and adult of both sexes weighing 110 to 160 g. were used. Two rats at a time were fed on polished boiled rice, carrot, cod liver oil and water *ad libitum*. After they were accustomed to this diet, one rat was given daily for a week 30 mg. of 4-dimethylaminoazobenzene dissolved by

heat in the cod liver oil. The other rat, used as control, was fed continuously on the rice-carrot diet without azo dye. Consecutively the rest of the animals, in couples, were fed as above. All the animals in the experiment were housed at 23° C. separately in screen-bottomed cages. The mortality of the animals treated with DAB was 40 per cent.

*Isolation of the soluble proteins of rat liver.*—The rats, after fasting for 16–20 hours, were killed with ether and in a cold room (0–5° C.) the livers were perfused for 10 minutes *in situ* with cold 0·15M NaCl through the vena porta. The livers were removed and a small fragment was taken away to ascertain qualitatively the approximate level of protein-bound dye in the liver as described below. Then the livers were quickly weighed and placed in a freezer at — 10° C. for 12–16 hours. After gradual thawing the liver was homogenized at 0° C. with a glass homogenizer with teflon plunger in 2 volumes of cold solution of 0·1M LiCl–0·04M $NaHCO_3$–0·01M $Na_2CO_3$. After 10 minutes it was centrifuged at 24,000 × g. for 30 minutes at 0° C. The sediment was extracted again for 10 minutes in 1 volume of the same extracting solution and was centrifuged as above. The supernatants were mixed and centrifuged at 0° C. at 24,000 × g. for 60 minutes. The final supernatants (total extracts) were clear amber from the control animals and brown from the animals fed on DAB.

*Separation of the total extract of liver soluble proteins.*—The total extract fractions were separated by means of salting-out using ammonium sulphate to 0·50 and 0·65 of saturation. The salting-out procedure was accomplished by dialysis at 5° C. for 24 hours against 10 volumes of the solution of ammonium sulphate buffered with phosphate at pH 7·3 replaced twice. The precipitates of the fractions at 0·50 and 0·65 of saturation were brought to the original volumes with cold solution of 0·15M NaCl–0·1M $NaHCO_3$.

*Bound dye analysis.*—In the liver the bound dye was revealed by fixing one millimetre thick cross sections of liver in a mixture of formalin, acetic acid, ethanol, and water in the proportion of 1 : 1 : 3 : 5 respectively. After 5 minutes the hardened and bleached sections were blotted and transferred to 10 per cent trichloroacetic acid. Five minutes after the sections ceased to float, they generally had assumed a maximum pink coloration due to the protein-bound dye (Miller, Miller, Sapp and Weber, 1949).

In total extracts, in fractions 0·50 and 0·65 of saturation, and in the final supernatants, the bound dye was analysed by adding to their aliquot an equal volume of 20 per cent trichloroacetic acid and then, after stirring, the mixture was centrifuged at 5000 r.p.m. for 10 minutes. The sediments which showed a characteristic pink coloration were those endowed with the protein-bound dyes.

*Determination of catalase activity.*—Catalase activity in total extracts, fractions, and final supernatants of the liver soluble proteins was determined after dialysis at 5° C. for 72 hours against 50 volumes of 0·15M NaCl–0·1M $NaHCO_3$ replaced every 12 hours and then centrifuged at 5000 r.p.m. for 10 minutes to remove the slight precipitate present. 1·5 ml. of the perfectly clear samples taken in examination, containing 100 μg. total N, was kept at 0° C. for 10 minutes then 5 ml. of 0·025M hydrogen peroxide buffered with phosphate at pH 7·4 was added. After 2–5 minutes at 0° C. the enzymatic reaction was stopped with 0·6 ml. of 9·37M sulphuric acid. Successively at room temperature 3 ml. of 2·4M trichloroacetic acid were added, shaking well. The solution was left to stand for 15 minutes and filtered. To 5 ml. of the clear filtrate 1 ml. of 0·903M potassium iodide was added.

After 15 minutes, the colour developed was measured at a wave length of 560 mμ. with a Beckman spectrophotometer and the colorimetric readings were related to a standard curve of hydrogen peroxide. The catalase activity was estimated from the amount of split of the hydrogen peroxide and was calculated subtracting the value of the tests from that of the hydrogen peroxide control.

*N and P analyses.*—The total N was assayed by direct Nesslerization and total P following the Allen's method (1940). The total extract, the fractions, and the final supernatants were previously dialyzed as described above in determination of catalase activity.

*Symbols.*—Total extract, fractions, and final supernatant of the liver soluble proteins from the DAB treated and control rats were abbreviated to te-DAB ; 0·50-DAB ; 0·65-DAB ; s-DAB and te-C ; 0·50-C ; 0·65-C ; s-C respectively.

## RESULTS.

*N and P content of the liver soluble proteins and their fractions.*—Table I summarizes the analytical data for the content of N and P of the te-DAB ; 0·50-DAB ; 0·65-DAB ; s-DAB compared to the te-C ; 0·50-C ; 0·65-C ; s-C.

TABLE I.—*μg. of N and of P/1000 μg. total N in the Different Fractions of the Soluble Proteins of the Liver in the DAB and Control Animals.**

| | N. | P. | N/P. |
|---|---|---|---|
| te-DAB | 1000·0 | 30·60±1·24 | 32·70 |
| 0·50-DAB | 490·0±7·57 | 10·70±0·57 | 45·70 |
| 0·65-DAB | 53·7±1·36 | 0·75±0·67 | 71·70 |
| s-DAB | 112·0±1·47 | 1·94±0·73 | 57·70 |
| te-C | 1000·0 | 31·90±1·46 | 31·40 |
| 0·50-C | 540·0±4·04 | 10·10±0·96 | 54·00 |
| 0·65-C | 132·5±1·44 | 1·29±0·36 | 102·00 |
| s-C | 89·6±0·92 | 1·04±0·92 | 86·00 |

* Average values of 12 experiments.

te-DAB = Total extract of the liver soluble proteins from the rats fed on DAB.
te-C = " " " " " " " " " control rats.
0·50-DAB = Fraction at 0·50 of saturation of the total extract from the rats fed on DAB.
0·50-C = " " 0·50 " " " " " " " control rats.
0·65-DAB = " " 0·65 " " " " " " " rats fed on DAB.
0·65-C = " " 0·65 " " " " " " " control rats.
s-DAB = Final supernatant of the total extract from the rats fed on DAB.
s-C = " " " " " " " control rats.

The data in Table I show that the amount of N recovered in the 0·50 and 0·65 fractions is lower in the DAB animals than in the control animals. This is especially relevant from the 0·65 fraction. On the contrary, the supernatant, which evidently consists of rather low molecular proteins, is considerably larger in the DAB animals. Also the P content indicates a shift from the 0·65 to the supernatant, although this is smaller than the corresponding shift of N. That indicates a considerable breaking-down of the proteins especially of the 0·65 fraction in the liver of the DAB treated animals.

*Localization of the protein-bound dyes.*

Using the salting-out procedure with ammonium sulphate, the protein-bound dyes in the liver soluble proteins of rats fed on DAB are localized in the fraction

precipitating at 0·65 of saturation. This is revealed by the pink colour that this fraction presents to the bound dye analysis. However, sometimes a very few protein-bound dyes can be found also in s-DAB. This is a further indication of the break-down of the proteins of this fraction to compounds of lower molecular weights. The 0·50-DAB never shows protein-bound dye.

*Behaviour of the catalase activity.*

In examination of catalase activity in the total extracts of liver soluble proteins, we have found that the te-DAB exhibits a lowering of the enzymatic activity as expressed by the split of hydrogen peroxide in 2 minutes of reaction at 0° C. Indeed the te-DAB gives values of catalase activity ranging between 625 $\mu$g. and 925 $\mu$g./100 $\mu$g. N of hydrogen peroxide break-down against 1300–1450 $\mu$g./100 $\mu$g. N of the te-C. The lowering of catalase activity of the te-DAB is 55·3 per cent. In experiments on fractions of both the DAB and control animals only the 0·50 fraction exhibits catalase activity which, however, is lower than that of the original total extract. Therefore it was estimated after 5 minutes of reaction instead after 2 minutes. This decrease of the activity can be ascribed to the adsorption of the catalase on the cellophane tubing during dialysis (Hennichs, 1926 ; Sumner, 1941). The 0·50-DAB fraction shows the same lowering of the catalase activity with respect to the corresponding fraction of the control animals just as it has been observed in the total extract. In fact, the activity of this fraction gives values 400–500 $\mu$g. in respect to 825–1250 $\mu$g. of the 0·50-C. The reduction in catalase activity of the 0·50-DAB is 43·9 per cent. Furthermore the fraction at 0·65 of

TABLE II.—*Catalase Activity in the Total Extracts and Fractions of the Liver Soluble Proteins in the DAB and Control Animals.**

| Exper. No. | te-C†. | te-DAB†. | 0·50-C‡. | 0·50-DAB‡. | 0·65-C‡. | 0·65-DAB‡. | s-C‡. | s-DAB‡. |
|---|---|---|---|---|---|---|---|---|
| 1 | 1400 | 900 (64·3) | 825 | 525 (63·5) | 105 | 125 | 0 | 0 |
| 2 | 1350 | 625 (46·3) | 1250 | 540 (35·2) | 80 | 115 | 0 | 0 |
| 3 | 1425 | 700 (49·1) | 1050 | 430 (40·9) | 105 | 120 | 0 | 0 |
| 4 | 1300 | 720 (55·4) | 1125 | 465 (41·3) | 95 | 125 | 0 | 0 |
| 5 | 1325 | 815 (61·5) | 980 | 510 (52·0) | 120 | 135 | 0 | 0 |
| 6 | 1450 | 925 (63·8) | 1100 | 400 (36·3) | 125 | 125 | 0 | 0 |
| 7 | 1410 | 820 (53·1) | 1150 | 425 (36·9) | 100 | 130 | 0 | 0 |
| 8 | 1315 | 780 (59·3) | 975 | 530 (34·4) | 115 | 135 | 0 | 0 |
| 9 | 1425 | 675 (47·4) | 1065 | 525 (49·3) | 85 | 125 | 0 | 0 |
| 10 | 1350 | 690 (51·1) | 1210 | 410 (33·9) | 80 | 120 | 0 | 0 |
| 11 | 1325 | 740 (55·8) | 1210 | 540 (44·6) | 100 | 115 | 0 | 0 |
| 12 | 1300 | 750 (57·7) | 1115 | 435 (39·0) | 90 | 130 | 0 | 0 |
| Average | 1364 | 761 (55·3) | 1087 | 469 (43·9) | 100 | 125 | 0 | 0 |

* In terms of $\mu$g. of hydrogen peroxide split at 0° C. by total extract and fraction containing 100 $\mu$g. total N.
† After 2 minutes' reaction.
‡ After 5 minutes' reaction.
The percentage diminution of the catalase activity is expressed in parentheses.

te-C = Total extract of the liver soluble protein from the control rats.
te-DAB = " " " " " " " " rats fed on DAB.
0·50-C = Fraction at 0·50 of saturation of the total extract from control rats.
0·50-DAB = " " 0·50 " " " " " " the rats fed on DAB.
0·65-C = " " 0·65 " " " " " " the control rats.
0·65-DAB = " " 0·65 " " " " " " the rats fed on DAB.
s-C = Final supernatant of the total extract from the control rats.
s-DAB = " " " " " " " rats fed on DAB.

saturation exhibits a very small catalase activity possibly due to the enzyme contamination. The catalase activity observed in these fractions is too weak for a comparison to be made between DAB and control animals. We never have found catalase activity in the s-DAB and s-C. The values of the catalase activity of the total extracts and fractions are shown in Table II.

## DISCUSSION.

To study the behaviour of the catalase activity in the first steps of the tumoural induction our animals have been given a higher amount of 4-dimethylaminoazobenzene (30 mg. daily) than usually employed. As is well known, the level of hepatic bound dye increases with increasing the dietary level of dye. In this way the protein-bound dye is easier to detect after a week, and at the same time the action of the carcinogenic agent is more powerful. The results we have obtained with the soluble proteins seem to demonstrate that the lowering of the catalase activity can be disclosed in the earliest stages during the induction of liver tumours only.

The catalase activity in rats fed on DAB has been investigated by Euler, Hasselquist and Eriksson (1952) and by Mori and Momoki (1952). They describe a decrease of catalase activity in the liver tissue of rats fed on 0·06 per cent DAB in the diet for a long period of time, whereas the activity of kidney catalase did not change. Our results agree with these findings. However, with our method of extraction and fractionation of the liver soluble proteins, we have succeeded in showing that the fraction to which the dye is bound is different from the one bearing catalase activity. Thus it appears likely that the amino azo dye does not act directly on the liver catalase and hence the decrease of its activity is probably related to some inhibitor called forth by the metabolic changes produced in liver by DAB. In this view the lowering of the catalase activity which we have observed in the first steps of the neoplastic induction may be regarded as similar to that occurring in large tumours.

Furthermore, the liver protein fractions of rats fed on DAB show considerable differences in N and P content and in N/P ratio compared to the control rats. The N and P content in fractions at 0·65 of saturation, where the protein-bound dyes are localized, is decreased in respect to that of the non-treated animals. The level of N and P is always more elevated in the supernatant of the treated animals. The N/P ratio of all the fractions and supernatant from rats fed on DAB presents a constant lowering in respect to the control animals.

These results suggest that the liver soluble proteins from rats treated with DAB appear as if they were broken down. In this way the lower precipitation of the soluble proteins at 0·50 and 0·65 of saturation and the larger amount of the non-precipitable proteins in the supernatant can be explained.

## SUMMARY.

1. The liver of rats both not fed on and fed on 30 mg. of 4-dimethylaminoazobenzene daily for one week were perfused, homogenized and the soluble, non-particulate proteins extracted. The total extracts were separated by salting-out, using ammonium sulphate at 0·50 and 0·65 of saturation. The total extract, the fractions at 0·50 and 0·65 of saturation, and the final supernatant were analysed for N and P content, protein-bound amino azo dye, and catalase activity.

2. Using the salting-out procedure the major part of the protein-bound dyes were localised in the fraction precipitated at 0·65 of saturation, whereas the fraction at 0·50 was endowed with the total catalase activity.

3. In rats treated with DAB the total extracts of the liver soluble proteins show a distinct lowering of the catalase activity, this lowering is also observed in fraction at 0·50 of saturation where all the original catalase is localized.

4. The content of N and P of the total extract and 0·50 fraction from rats fed on DAB was comparable to those of control rats. The fraction at 0·65 of saturation in which occur the protein-bound dye but not the catalase, presents a considerable decline of N and P in animals fed on DAB in respect to the controls. On the contrary the amount of N and P of the final supernatant is higher in DAB treated rats than in controls. These changes of the N and P content were regarded as expression of the metabolic alteration caused by the carcinogen on the liver.

5. The lowering of the catalase activity seemed to refer to an anticatalase substance, brought about by the metabolic changes which occur in the liver during the neoplastic induction more than to a direct action of the azo dye upon the catalase, since the catalase and the aminoazo dye were localized in different fractions. Perhaps the phenomenon of the fall of the liver catalase activity in tumour-bearing animals arises already in the first steps of the neoplastic induction, but it cannot be early detected in tumours far from the liver because the tumoural anticatalase substance is neutralized in the blood stream or in some tissues before reaching the liver.

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## REFERENCES.

ABRIGNANI, F. AND MUTOLO, V.—(1954) *Experientia*, **10**, 470.
ADAMS, D. H.—(1954) *Brit. J. Cancer*, **7**, 501.
ALLEN, R. J. L.—(1940) *Biochem. J.*, **34**, 858.
COLWELL, A.—(1910) *Arch. Middx. Hosp.*, **19**, 64.
EULER, H., VON, HASSELQUIST, H. AND ERIKSSON, E.—(1952) *Arkiv. Kemi*, **4**, 487.
GREENSTEIN, J. P.—(1943) *J. nat. Cancer Inst.*, **3**, 397.—(1954) 'Biochemistry of Cancer.' New York (Academic Press).
HARGREAVES, A. B. AND DEUTSCH, H. F.—(1952) *Cancer Res.*, **12**, 720.
HENNICHS, S.—(1926) *Biochem. Z.*, **171**, 314.
KENSLER, C. J., SUGIURA, K., YOUNG, N. F., HALTER, C. R. AND RHOADS, C. P.—(1941) *Science*, **93**, 308.
LUCKÉ, B. AND BERWICK, M.—(1954) *Proc. Amer. Ass. Cancer Res.*, **1**, 30.
*Iidem* AND ZECKER, I.—(1952) *J. nat. Cancer Inst.*, **13**, 581.
MAGAT, J.—(1924) *Z. ges. exp. Med.*, **42**, 95.
MCDONALD, J. C., PLESCIA, A. M., MILLER, E. C. AND MILLER, J. A.—(1953) *Cancer Res.*, **13**, 292.
MILLER, E. C. AND MILLER, J. A.—(1947) *Ibid.*, **7**, 468.—(1952) *Ibid.*, **12**, 547.—(1953) *Advances in Cancer Res.*, **1**, 339.
*Iidem*, SAPP, R. W. AND WEBER, G. M.—(1949) *Cancer Res.*, **9**, 336.
MORI, K. AND MOMOKI, S.—(1952) *Gann.*, **43**, 431.

MUTOLO, V. AND ABRIGNANI, F.—(1954) *Boll. Soc. ital. Biol. sper.*, **30**, 209.
NAKAHARA, W. AND FUKUOKA, F.—(1950) *Gann.*, **41**, 47.
PRICE, J. M., MILLER, J. A., MILLER, E. C. AND WEBER, G. M.—(1949) *Cancer Res.*, **9**, 96.—(1949) *Ibid.*, **9**, 398.
ROSENTHAL, E.—(1912) *Dtsch. med. Wschr.*, **38**, 2270.
SOROF, S. COHEN, P. P., MILLER, E. C. AND MILLER, J. A.—(1951) *Cancer Res.*, **11**, 383.
*Idem*, GOLDER, R. H. AND OTT, M. G.—(1954) *Ibid.*, **14**, 190.
STEVENSON, E. S., DOBRINER, K. AND RHOADS, C. P.—(1942) *Ibid.*, **2**, 160.
SUMNER, J. B.—(1941) *Advanc. Enzymol.*, **1**, 163.
YOUNG, N. F., COLLIER, V. AND HOMBURGER, F.—(1947) *Proc. Soc. exp. Biol., N.Y.*, **66**, 323.
WEIL-MALHERBE, H. AND SCHADE, R.—(1948) *Biochem. J.*, **43**, 118.

# CYTOPLASMIC SYNTHESIS IN NERVE CELLS.

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THE chromatolytic cycle occurring in the nerve cell body (perikaryon) following removal of most of the cytoplasm by section of the nerve processes has been studied by a host of workers during the past century. The use of chromatolysis to map out the topographical distribution of nuclei and their connnections does not directly concern us here ; rather are we concerned with the parallel study of the detail of the cytological changes during the chromatolytic cycle. This concerns us firstly as an approach to the general problem of cytoplasmic synthesis and restitution, since here we have an unrivalled opportunity to study a cell from which the bulk of its cytoplasm has been removed ; secondly because the nerve cell body presents a specialised problem in protoplasmic synthesis ; its axoplasm is so extended, and so large in bulk, that the portion of the cell concerned with maintenance of cytoplasmic integrity is presented with a greater load than any other cell. The maintenance of the axon is of particular interest in view of the rather specialised reaction of nerve cells to infective and neoplastic processes. The effect of axon section on the cytoplasm of the neurones was described by Nissl (1892) and since that time the process of disintegration of the chromophil material of the nerve cell has been described as chromatolysis and its reconstitution as chromosynthesis.

The time of onset of chromatolysis and of recovery therefrom varies according to the type of injury, the site of injury and the type of cell involved, but the general order of these events can be summarised : latent period of 1 or 2 days before break up of the Nissl substance begins ; degradation of the Nissl substance is at a maximum between the 1st and 2nd weeks after axon interruption ; chromosynthesis begins in the 1st to 3rd week and may be completed by one month or may be delayed for several months (Bodian and Mellors, 1945).

Biochemical aspects and relationships of Nissl substance are discussed in three reviews in the Society of Experimental Biology Symposium on nucleic acids (1947) respectively by Caspersson, Hyden and Bodian. The detailed description of the structure of Nissl substance, as revealed by electron microscopy, has been given by Palade (1954). He finds that the basophilic element in cytoplasm (ergastoplasm) consists of double-walled vesicles or canals (the endoplasmic reticulum) surrounded by electron dense granules of approximately 120 A.U. diameter. Nissl substance was found to consist of vesicles with granules, together with dense aggregates of these granules in between. One clump of these constitutes a classical Nissl granule.

Mitochondria (chondriomes) have in general been considered as much more constant cellular elements, but very labile in regard to their reactions to fixatives.

The definition of a mitochondrion in classical microscopy has been based mainly on shape, size, vital staining, and reaction to fixatives. Palade's (1952*a*, 1953) electron microscopic studies, together with those of Sjöstrand and Rhodin (1953) revealed the internal structure—a double-layered limiting membrane of about 160 A.U. spacing, with internal cross membranes, usually in the plane of the short axis, also double-layered, and called by Palade the " cristae mitochondriales ". There is evidence to support the production of mitochondria from cytoplasm lacking differentiated elements, and also for their propagation by division (de Robertis, Nowinski and Saez, 1954).

The present work is concerned with the structural changes shown by electron-microscopic methods in dorsal root ganglion cells of the rat during the period of activity occurring in the weeks following section of the nerves distal to the ganglion.

## MATERIAL.

The 4th and 5th lumbar nerves were sectioned on one side of the rat, in the sciatic plexus, by a lateral dorso-lumbar approach. After periods of 4, 7, 10, 14, 21 and 32 days the animals were sacrificed and the 4th and 5th dorsal root ganglia of both operated and unoperated sides were removed for examination. Ganglia from unoperated animals were used as controls. All animals were anaesthetised with nembutal, 35 mg./kg.

The ganglia were fixed in 1 per cent osmium tetroxide, buffered to approximately pH 7·4 (Palade, 1952*b*), for 4 hours at + 4° C., embedded in butyl methacrylate (polymerised at room temperature by U.V. light) and cut at approximately 2–300 A.U. on a Cook and Perkins thermal expansion microtome. The sections were examined in a Metropolitan-Vickers E.M.4. microscope working at 50 k.V., using a 50 $\mu$ objective aperture and photographed at about 5000 × primary magnification.

## NORMAL MATERIAL.

As pointed out by Nageotte (1932) the cells in normal ganglia show considerable variation in the appearance of their cytoplasm ; this is illustrated in Fig. 1, a light-microscopic picture of a group of dorsal root ganglion cells. The ganglion was fixed in exactly the same manner as for electron-microscopy, sectioned at 5 $\mu$, the methacrylate dissolved out with carbon tetrachloride, and the section stained with cresyl violet. As may be seen, the size and density of the Nissl granules vary considerably from cell to cell. Apart from large, discrete granules, the density of background staining of cytoplasm varies considerably. A few myelinated fibres may be seen between the cells, and the closely adhering satellite cell capsule is shown.

In Fig. 2 an electron micrograph of the junctional region between four normal nerve cells is shown (magnification approximately 30 times that of Fig. 1). The shrinkage space between the cells is seen to be extremely small, and in some of the intercellular spaces (ranging from 0·25–0·05 $\mu$) collagen bundles are prominent. The relationship between the satellite cells and the variable layers of cytoplasm with which they encapsulate the nerve cells are clearly seen : the satellite cells are bounded by double-layered membranes (of somewhat more than 150 A.U. spacing) so that wherever two satellite cells meet a quadruple layering results, but it seems

probable that this membrane is reduced between satellite cell and neurone ; sets of the membranes at higher magnification are shown in Fig. 3.

It will be observed that all four cells in Fig. 2 are rather similar in cytoplasmic composition ; all contain large and small aggregates of Nissl substance, while round and oval mitochondria are scattered throughout each cell. In the cell on the lower right a portion of the nucleus may be seen, with its dense nucleolus.

The fine structure of the cytoplasm in a densely granular cell is more clearly illustrated in Fig. 4. Scattered about the field are numerous formed mitochondria, showing cristae, whilst the Nissl substance varies from large, dense clumps of granules to relatively small aggregates, arranged around their central vesicles. As may be seen, granules commonly aggregate in rosettes and circlets of 4–6 in number. Vesicles are less common in the denser clumps of granules.

Typical mitochondria, of lengths from 0·5–1 $\mu$, are shown in Fig. 5, they are surrounded by a double-layered limiting membrane. Parallel double-lined cristae cross them almost perpendicular to the long axis. The spacing of laminae in limiting membrane and cristae is similar to that of the cell membrane.

The character of the cytoplasm of cells in the ganglia from the control side of operated animals was more variable than that of ganglion cells from normal animals. Such findings are not unexpected in view of the vascular changes following operation and alterations in postural activity.

## CELLS OF OPERATED GANGLIA.

In considering the changes that occur in cytoplasmic and nuclear structures after cutting the axons, a strictly chronological order will not be attempted because, as might be expected, all the cells in any one ganglion do not follow the identical cycle, nor the same time-course ; in fact some of the cells probably never recover. One such cell is shown in Fig. 6, where it will be seen that cytoplasmic elements are reduced to a minimum, and there is no evidence of that restorative activity which will later be shown to be evident in most of the cells. Such cells, presumably dying, have been rare in the material examined.

After allowing an interval of 21 days between section and sacrifice the picture seen in Fig. 7 was common, with eccentric nucleus and a nuclear membrane manifesting activity. No mitochondria or liposomes are seen, the cytoplasm being packed with large vesicles—some of which are beginning to show invagination of the limiting membranes—and Nissl substance is reappearing, mainly in the form of groups of granules around small vesicles.

The folding of the nuclear membrane in chromatolytic cells is a regular observation, and the most active phase is illustrated in Fig. 8, 9, 10, and 11 ; it is most striking after the 7th day. Fig. 8 and 9 are photographs of two sections, fairly close together, from the same cell ; they and Fig. 10 are from cells 14 days after axon section. The impression obtained from these corrugated nuclei is one of the throwing out of processes and the formation of large numbers of vesicles in relation to these processes. In Fig. 10 we see a dense nucleolus, double-layered nuclear membrane, and on two of the prominences are vesicles which seem still attached to the nuclear membrane. One of these seems to consist of a chain of vesicles, closely attached to each other and the nuclear prominence. This portion of the nuclear membrane is shown at larger magnification in Fig. 11, where another feature of

such nuclear membranes becomes clear ; the aggregation of dense material on the inner surface of the nuclear membranes, close to prominences.

In Fig. 8 and 9 there are rows of vesicles radiating out from the nuclear membrane, some of these vesicles may be seen to have a double limiting membrane, and most have a clear central part with electron dense aggregations near the surface. Many of the vesicles are apposed or attached to promontories on the nucleus, but others are associated with hollows in the nuclear membrane. Several of the vesicles may be identified in both sections (Fig. 8 and 9,), but the cytoplasmic pattern is noticeably different. Fig. 12 represents an enlarged region from Fig. 8 and shows one of the string-like attachments extending from the nuclear promontory towards a vesicle complex. This " string " is discontinuous in Fig. 9.

From 32-day material we obtained pictures suggestive of the completion of the cycle : the nuclear membrane is becoming smooth in outline, dense mitochondria are now spreading out into the cytoplasm and in between them, masses of Nissl substance are appearing (Fig. 13–16).

## MITOCHONDRIA.

In the examination of the material described above the formation of large vesicles in relation to an activated nuclear membrane has been indicated, and the occurrence of a ring of mitochondria around the nucleus has been demonstrated and interpreted as representing a stage in the recovery of the nerve cell from trauma. It would seem that there are many intermediate forms between these two stages.

Fig. 13, 14 and 15 show part of the cytoplasm of a cell 32 days after axon section, and illlustrate the many stages that occur between the large open vesicle that is seen in parts of Fig. 13 and the formation of cristae which are shown in greater detail in the enlargements of the areas A and B illustrated in Fig. 14 and 15.

The further increase in density of the newly-formed mitochondria is illustrated in Fig. 16. There are in the cytoplasm of the cell many dense bodies, still transected by numerous cristae, but with much dense material between cristae. There are a few typical mitochondria, one such being indicated in the inset, practically the whole of it is transected by cristae. Most of the mitochondria, however, have increased their overall density by the elaboration of material between the cristae. An example of this is shown in the second inset, where cristae can be seen only faintly outlined against a dense background.

The series of transitions shown could illustrate the process whereby a typical mitochondrion, with conspicuous parallel cristae and little granular substance between them, proceeds to elaborate osmiophilic material between the cristae, until the total density of the body is such as to obliterate the cristae. It would appear that the final stage in the evolution of these cytoplasmic organelles may be the formation of osmiophilic liposomes.

## DISCUSSION.

In assessing the significance of the observations that have been summarised in this paper the first point that presents itself is the problem of artefact. Sjöstrand and Hanzon (1954) in a study of the cytoplasmic inclusions in the mouse pancreas were not able to show any definite change in the ultrastructure following the injec-

## DESCRIPTION OF PLATES.

FIG. 1.—An optical photograph of a group of normal ganglion cells, stained with cresyl violet. Note the variation both in background staining and Nissl granules in the different cells. In the capsule of the large cell on the upper right a myelinated fibre can be seen.

FIG. 2.—Electron micrograph of a junctional region between four normal ganglion cells: all four cells are rich in formed mitochondria and Nissl substance. Closely adherent to each nerve cell is the cytoplasm of the satellite cells. In the lower left centre the point of contact between several satellite cells is to be seen: this is shown in further detail in Fig. 3.

FIG. 3.—A magnified view of a group of cell membranes from Fig. 2. Note the multiple sets of double-layered membranes, with spacing between the membrane lamellae of about 160–180 A.U.

FIG. 4.—A field of typical cytoplasm in a normal ganglion cell: on the right is dense Nissl substance, composed of small groups of granules, the individual members of which are around the 120 A.U. diameter range. In the lower part of the field, and on the left, there are smaller aggregations of granules, sometimes arranged around vesicles and typical mitochondria.

FIG. 5.—Typical mitochondria from a ganglion cell. These range from 0·25 to 1 $\mu$ in their long axis, and are transected approximately perpendicular to it by double-layered cristae. The spacing in the layers of these cristae is about 150 A.U.

FIG. 6.—A very abnormal cell from a ganglion removed 10 days after operation. The nucleus can be seen surrounded by cytoplasm almost completely emptied of its organelles.

FIG. 7.—A cell from a ganglion 21 days after operation. The nuclear membrane is still somewhat folded and the cytoplasm is packed with simple, hollow vesicles. Some of these are still in close association with the nuclear membrane, but the larger vesicles are becoming separated by small vesicles and their associated rings of tiny granules. A little material may be seen within the vesicles and some of them are commencing invagination.

FIG. 8 and 9.—These two figures are photographs at the same magnification of similar regions in two serial sections of the same cell. The nuclear membrane is folded. In Fig. 8 a strand of material extends from a triangular projection on the left towards a complex group of small vesicles; this is shown enlarged in Fig. 12. In Fig. 9 this strand is absent. Some of the hollow vesicles in Fig. 8 may also be identified in Fig. 9. A small vesicle may be seen closely adherent to a hollow in the nuclear membrane in Fig. 9: (↑) this vesicle may also be identified in Fig. 8. The mass of the cytoplasm of this cell is packed with hollow vesicles; no Nissl substance can be seen. From a ganglion 14 days after section of the nerve roots.

FIG. 10.—Portion of a cell from 14-day operated material. The nuclear membrane is seen to be markedly folded and from two of the projections, vesicles appear to be separating off. In these two regions the vesicles are still attached to the nuclear membrane. Throughout the cytoplasm chains of vesicles of various sizes may be seen; in some of them the two layers of membrane are visible. The spacing of the layers is about 150 A.U.

FIG. 11.—An enlarged view of portion of the nuclear membrane, and associated vesicles, from Fig. 10. The double layering of the nuclear membrane and the attachment of the vesicles is shown. Dense aggregation can be seen on the nuclear membrane.

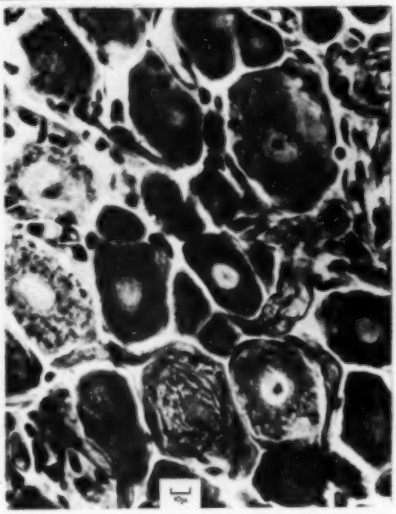
FIG. 12.—An enlarged portion of Fig. 8. The string-like prolongation of the nuclear membrane (↑) is seen to extend to a tightly associated group of vesicles.

FIG. 13.—Portion of a cell from 32-day material. All stages between hollow double-layered vesicles and fully formed mitochrondia may be observed. The small vesicles and associated granules and dense granular aggregations pack the spaces between the large vesicles and mitochondria.

FIG. 14.—A higher magnification of portion of Fig. 13. Here an early stage in invagination in a vesicle may be seen in the upper centre; two cristae extend into the lumen. In the upper right is a simple vesicle, while in the lower left is an almost typical mitochondrion with short cristae.

FIG. 15.—A second magnified portion from Fig. 13. Approximately in the centre is a half-formed mitochondrion, with three regions remaining electron-transparent. Numerous cristae transect it. To the left is another body undergoing invagination, with several short invaginating buds invading a hollow centre.

FIG. 16.—Portion of a cell from 32-day material. A mitrochondrial ring in the perinuclear position is seen, but the component organelles are spreading out into the peripheral cytoplasm and some Nissl substance is appearing between them. Although the cristae are still clearly visible in many of the organelles, their overall density is increasing from laying down of material between the cristae. This is shown by comparing the body seen at *a* (enlarged in inset *a'*) where the cristae are clearly defined, with that seen at *b* (enlarged in inset *b'*) in which the cristae may be faintly detected against a dense background.

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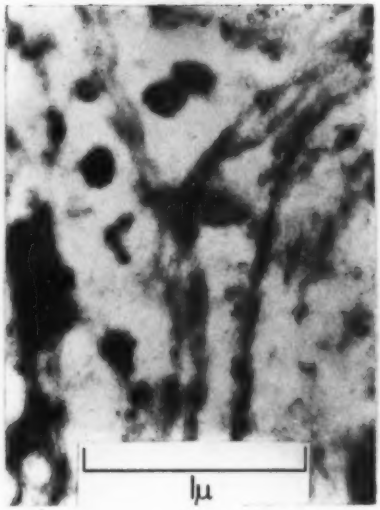


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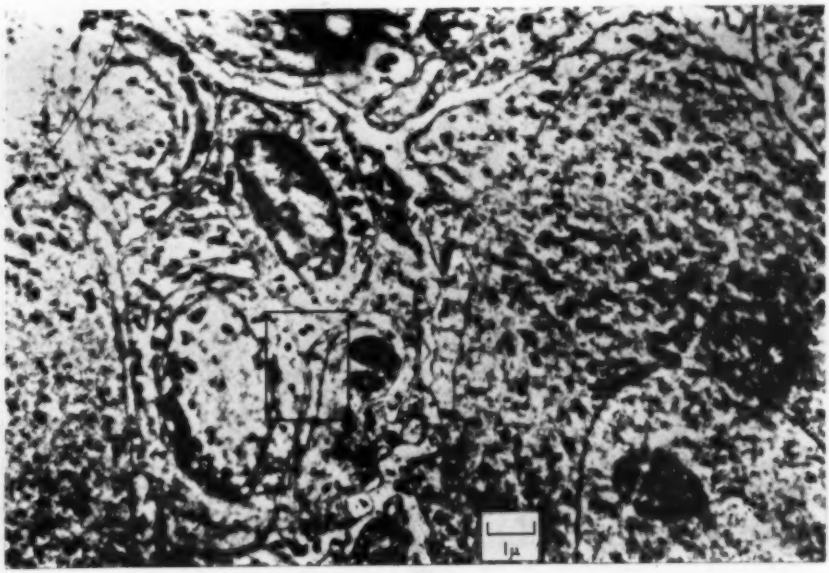


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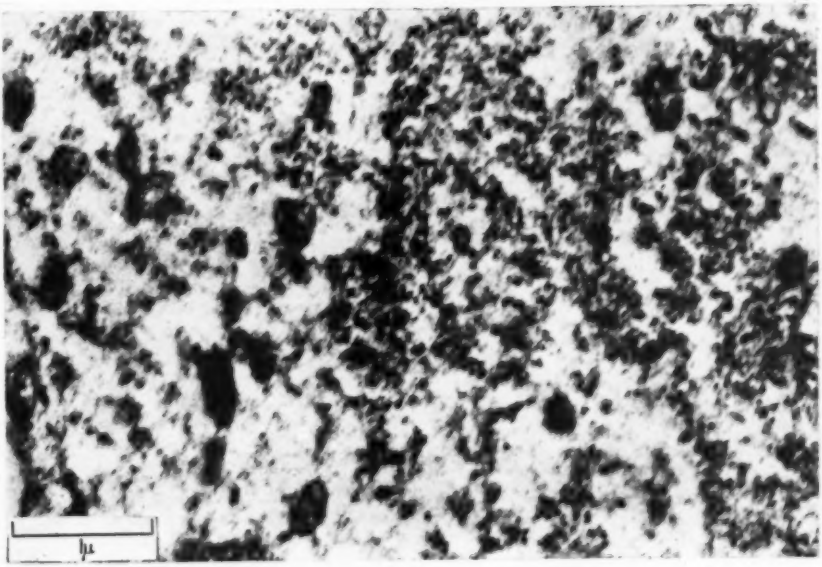


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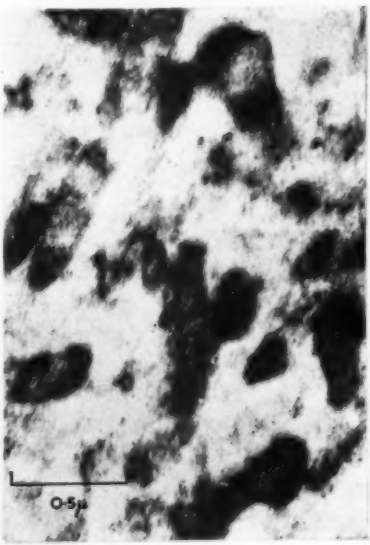


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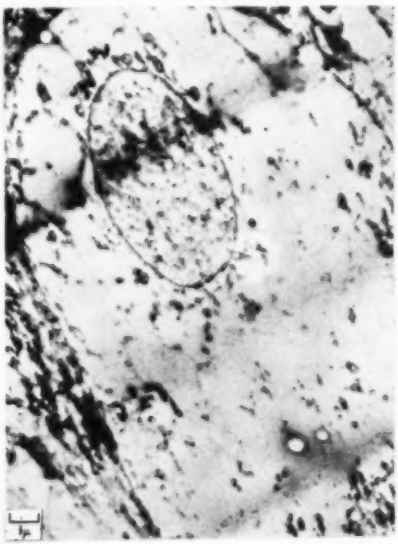


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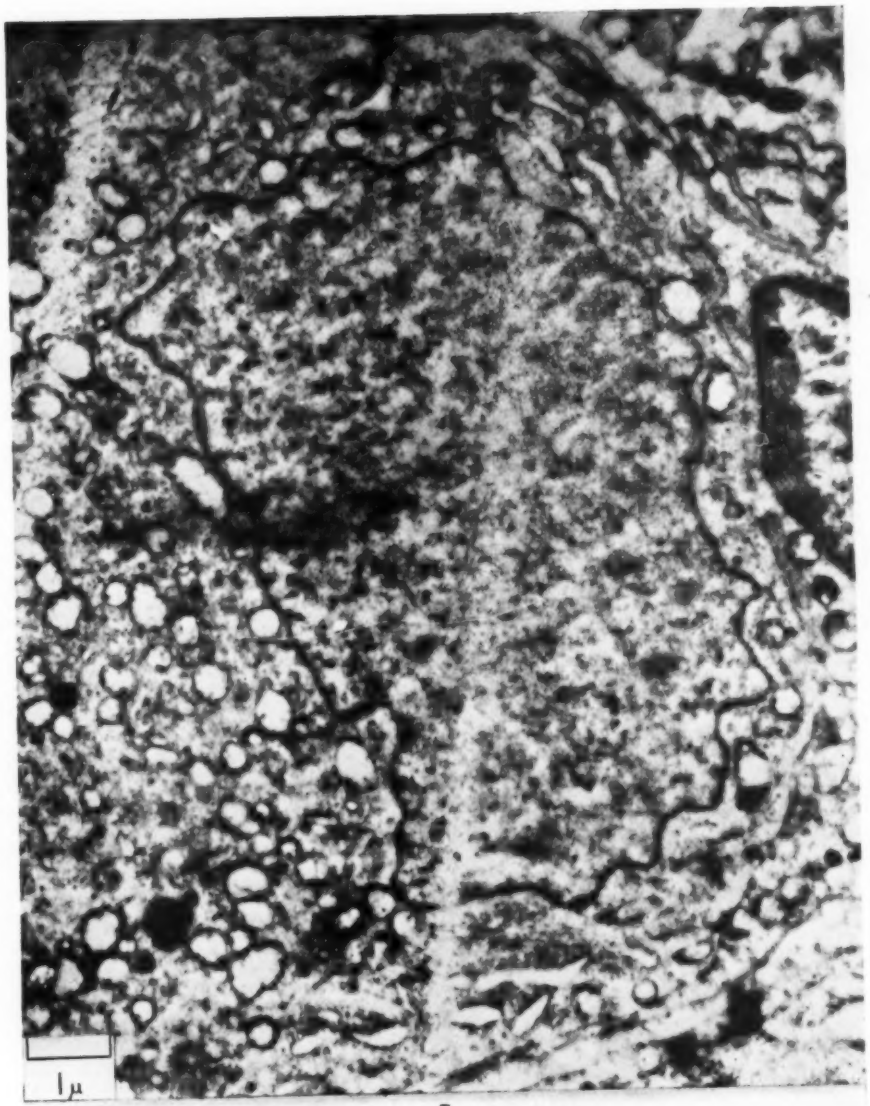


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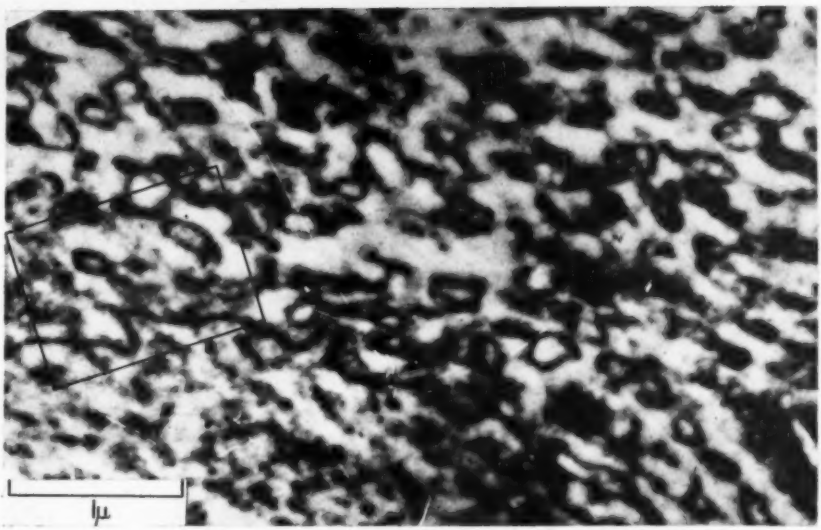


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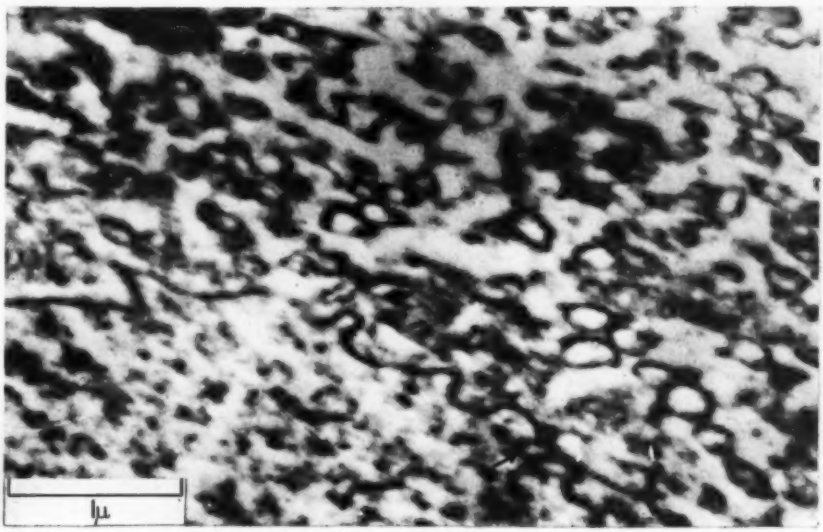


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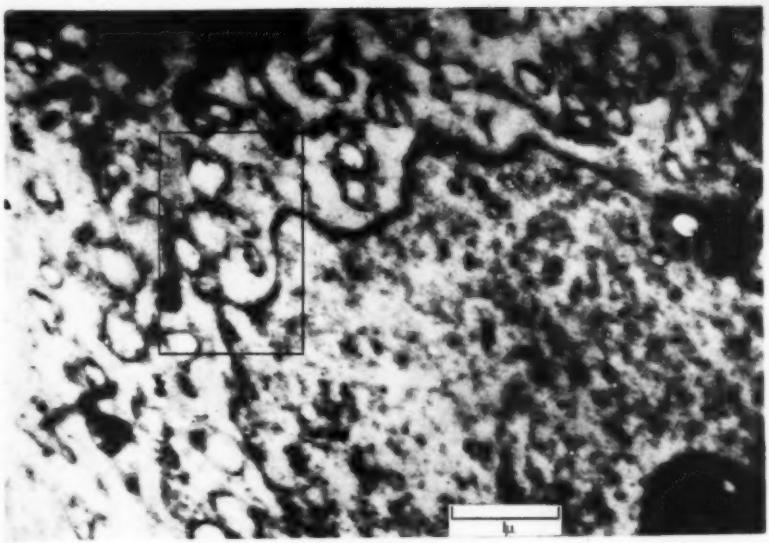


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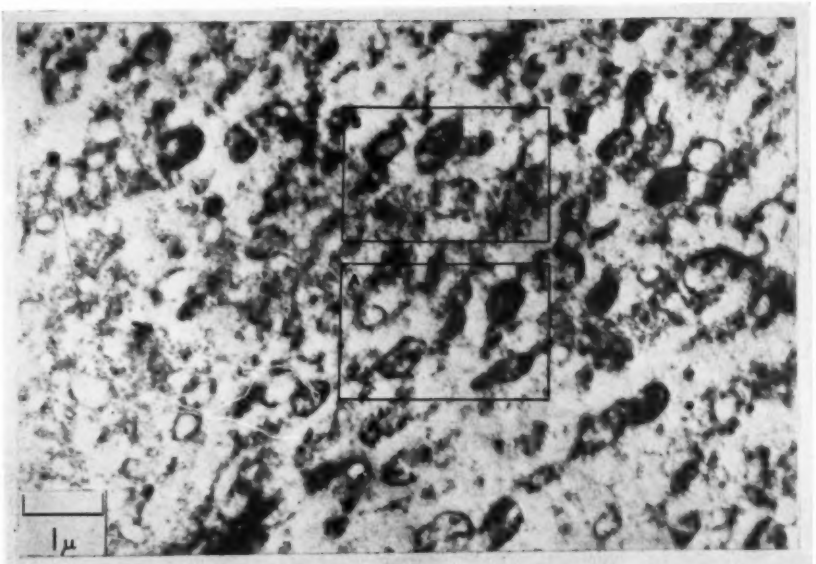


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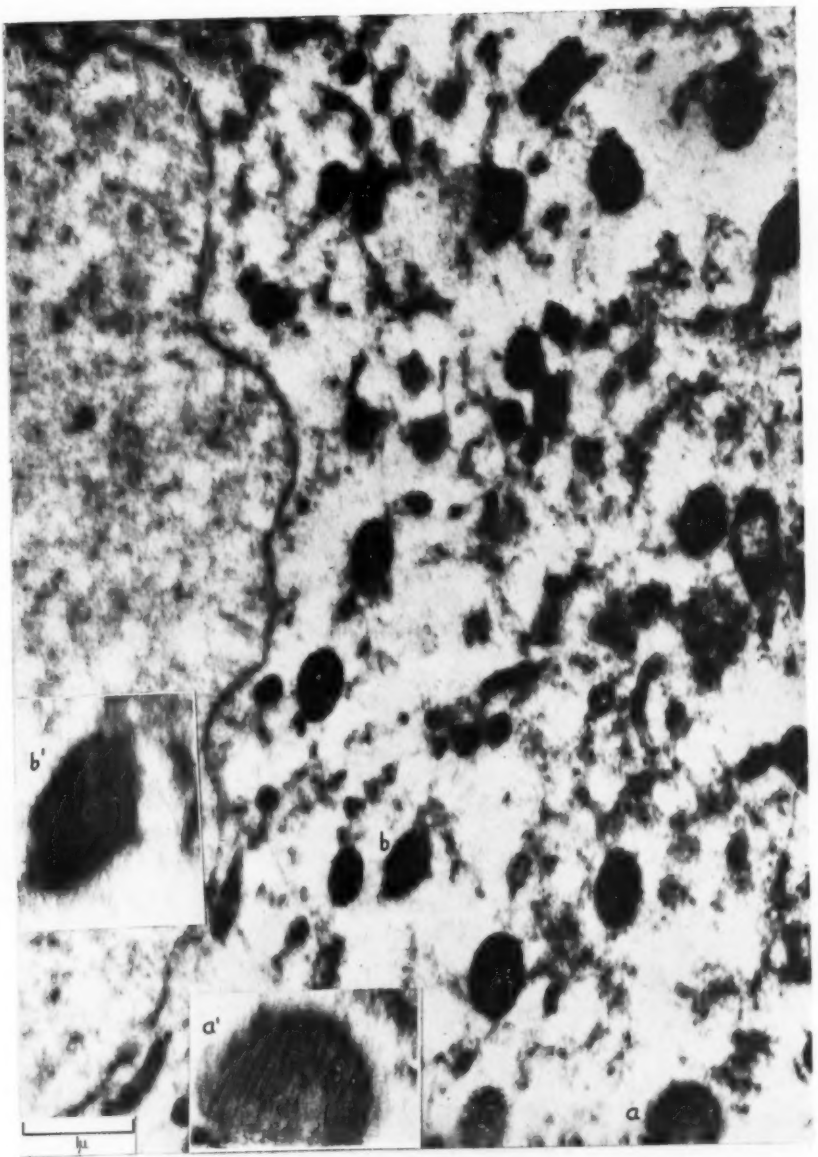


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tion of pilocarpine or after starvation, and draw attention to the drastic structural changes following improper fixation. We have found reproducible changes in ganglion cells after section of the nerve roots. Whilst we agree that very careful fixation is an essential it does seem legitimate to consider that with such fixation, making due allowance for variation between different specimens and checking these against the appearances in different parts of the same specimen, it is justifiable to draw conclusions from the changes in what must at worst be the same artefact, i.e. the artefact produced by buffered osmium tetroxide, or at best to consider this fixative as giving a fair reproduction of the structure in the living cell.

Of the three main structures discussed the nucleus and mitochondria are in accord with the phase contrast appearances in living cells and the Nissl substance fits well with the optical picture. The more general features of the chromatolytic cycle, such as eccentricity of the nucleus and gross depletion of a few cells, is again shown in this investigation. As the section of the nerve roots was close to the ganglia it would be expected that the changes are of a marked type (Heidenhain, 1911). The actual changes in cell size have not been studied.

The Nissl substance can be seen to go through a cycle of changes that is apparently similar to the changes recorded optically, but the individual Nissl granule of the optical picture is the aggregated clump of granules of macromolecular size. The loss of these aggregates is first seen in relation to the areas around the nuclear membrane and the dispersion of the larger aggregates such as are seen in Fig. 2, 4 and 6. It seems probable that this loss of aggregation is comparable to the stage of " dust like " particles in the classical optical picture. In material 32 days after section there is evidence of the reformation of the aggregates, but it is difficult to assess their origins. Many of the large vesicles that are found in association with the activated nuclear membrane show areas of granular and small vesicle clumps around their surfaces which might be the precursors of the Nissl material. In the description of the material two major classes of vesicle have been distinguished. The small vesicle, associated with the Nissl substance, having a diameter ranging around 500 A.U. to 1000 A.U., and the large vesicle with a diameter between 0·25 and 1·0 $\mu$ which has been associated with the mitochondrion. The relationship between the two groups is uncertain.

The mitochondria can be traced through a fairly continuous series of characteristic forms. The mitochondria of mammalian nerve cells after injury are said to be relatively stable in structure (Clarke, 1914 ; Strongman, 1917 ; McCann, 1918), but Luna (1913) described mitochondrial changes during the chromatolytic cycle in the toad. In our examination of the mitochondria they show marked changes in structure during the activity induced by section of the cell processes. It is not possible in electron microscopic studies to classify these inclusions according to their affinity for vital dyes, but the ability to define the internal structure makes it reasonable to accept the description and definition of " normal " mitochondria given by Palade (1952*a*)—the elongated body with transverse cristae. The evidence is that there are all sorts of intermediate forms between the " normal " mitochondrion defined above and the large double walled vesicles.

The internal structure of the mitochondrion was described in detail by Palade (1952*a*), who regarded the cristae mitochondriales as an infolding of the inner of the two dense laminae of the mitochondrial membrane. Sjöstrand and Hanzon (1954) do not accept the interpretation offered by Palade, they conclude that the membranes inside the mitochondrion are " individual structures with only topo-

graphic relations to the outer membrane ". Our material supports the interpretation of Palade ; the first evidence of cristae in a vesicle appears as infoldings around the periphery of the large vesicles ; the cristae seem to be associated with the inner dense layer of the surface, but our evidence on this point is not critical.

Considerable interest attaches to the relationship between the group of large vesicles and the nuclear membrane. Those cells that show the most cytoplasmic change after section of the nerve processes also show folding of the nuclear membrane. The appearances of the nuclear membrane are those of activity. There is a bulging-out of the membrane into hillocks with intervening valleys that is particularly well shown in the serial pictures. In relation to these evaginations there are in many cases rows of large vesicles. It would seem that the large vesicles are either pseudopodial processes that have been constricted off or are at least structures formed in close association with an activated nuclear membrane. Many of these vesicles show connections with the promontories on the nuclear membrane. That these vesicles then form condensations within their walls and invaginations of their wall to form a typical mitochondrion seems indicated. It is possible that further deposition of electron dense material lead to the formation of a typical liposome.

The absence of Nissl substance and mitochondria from the cytoplasm of nerve cells may be noted in a small proportion of the cells of normal ganglia, as pointed out earlier. This loss of Nissl substance and mitochondria is, however, so common in the middle period of chromatolysis as to affect the majority of the cells and gives an electron micrographic picture that may be considered equivalent to the classical chromatolytic picture of optical microscopy. There is evidence that practically all the enzymes of the cytochrome-cytochrome oxidase system are concentrated in mitochondria (Hogeboom, Claude, and Hotchkiss, 1946) and the work of Howe and Mellors (1945) established a fall in cytochrome oxidase in spinal cord during the chromatolytic period. Therefore the fate of the mitochondria of nerve cells during early stages of chromatolysis is of especial interest : if such bodies are formed from (or at) the nuclear membrane, it follows that they constitute one of the elements of the axon or dendrite which must be supplied from the perikaryon. It is possible that in the period immediately following section the mitochondria, and other formed elements of the cell body, are poured out into the cell process. The few depleted cells presumably represent individuals which have lost their organelles and are incapable of restitution.

The increasing density of certain of the mitochondria appears to be due to the laying down of electron-dense granular material between the cristae. That this material owes its density to osmiophilia is corroberated by the fact that thicker sections, cut from the same ganglion; are seen in the light microscope to possess typical osmiophil spheres in the same justanuclear position as these typical liposomes seen in the electron microscope. Practically every such body still betrays its origin in electron micrographs by the presence of dense cristae, faintly outlined against the surrounding material.

Most of the stages in the formation of vesicles and mitochondria, so evident in the operated ganglia, may be detected to a lesser degree in normal ganglia. Many simple vesicles, and various stages in the invagination to form mitochondria, have been observed. This would suggest that the processes of cytoplasmic synthesis and restitution are proceeding at various rates in the cells of normal ganglia, and that the response to axon section is merely an acceleration of this process. Our observations on Nissl substance, mitochondria and the nuclear membrane strongly

support Bodian's (1947) statement: "Regeneration after interruption of the adult axon can be viewed not only as a reversion to embryonic activity but also as an artificially accelerated normal function."

SUMMARY.

1. The electron microscopic appearances of the dorsal root ganglion cells of the rat are described and illlustrated.

2. The cytoplasmic changes during the cycle of chromatolysis and chromosynthesis are described.

3. During the cycle activation of the nuclear membrane is seen and the formation of vesicular structures that can be traced in different cells through stages of incomplete crista formation to formed mitochondria and probably from formed mitochondria to liposomes.

4. The disintegration of Nissl substance has been followed and its reappearance observed.

5. The relation of these observations to the possible course of cytoplasmic synthesis in nerve cells is discussed.

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## REFERENCES.

BODIAN, D.—(1947) *Symp. Soc. exp. Biol.*, No. 1, p. 163.
*Idem* AND MELLORS, R. C.—(1945) *J. exp. Med.*, **81**, 469.
CASPERSSON, T.—(1947) *Symp. Soc. exp. Biol.*, No. 1, p. 127.
CLARKE, E.—(1914) *J. comp. Neurol.*, **24**, 61.
DE ROBERTIS, E., NOWINSKI, W. W. AND SAEZ, F. A.—(1954) 'General Cytology.' Philadelphia and London (W. B. Saunders & Co.). 2nd ed., pp. 123–4.
HEIDENHAIN, M.—(1911) 'Plasma und Zelle.' Jena (G. Fischer).
HOGEBOOM, G. H., CLAUDE, A. AND HOTCHKISS, R. D.—(1946) *J. biol. Chem.*, **165**, 615.
HOWE, H. A. AND MELLORS, R. C.—(1945) *J. exp. Med.*, **81**, 489.
HYDEN, H.—(1947) *Symp. Soc. exp. Biol.*, No. 1, p. 152.
LUNA, E.—(1913) *Anat. Anz.*, **44**, 413.
MCCANN, G. F.—(1918) *J. exp. Med.*, **27**, 31.
NISSL, F.—(1892) *Allg. Z. Psychiat.*, **48**, 197.
NAGEOTTE, J.—(1932) 'Cytology and Cellular Pathology of the Nervous System.' New York (Hoeber). Vol. 1.
PALADE, G. E.—(1952*a*) *Anat. Rec.*, **114**, 427.—(1952*b*) *J. exp. Med.*, **95**, 285.—(1953) *J. Histochem. Cytochem.*, **1**, 188.—(1954) *Congr. int. Microscop. électron.* (in press).
SJÖSTRAND, F. S. AND HANZON, V.—(1954) *Exp. Cell Res.*, **7**, 393.
*Idem* AND RHODIN, J.—(1953) *Ibid.*, **4**, 426.
STRONGMAN, B. T.—(1917) *Anat. Rec.*, **12**, 167.

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# SURVEY OF PAPERS

STOCKS has classified the deaths from cancer of the cervix in England and Wales in 1950–51 according to age, marital condition and, in the case of married women, the social class of the husband and whether or not there had been any children. Among his conclusions are that the death rate is much higher for childless married women than for single women, and also that early marriage is a more important factor than number of confinements (p. 487).

KREYBERG presents further Norwegian figures supporting the belief that there is an association between cigarette smoking and the occurrence of certain histological types of lung cancer (p. 495).

GALLUZZI and PAYNE discuss the factors which determine the incidence and distribution of metastases in patients with bronchial carcinoma, as shown in the records of 741 necropsies relating to carcinoma of the bronchus from eight London hospitals (p. 511).

KEEN, DE MOOR, SHAPIRO and COHEN find that, whereas lung cancer is relatively infrequent in the South African Bantu, there is a remarkably high incidence of carcinoma of the nose and paranasal sinuses in this race. They suggest that this might well be accounted for by their extensively practised habit of snuff taking. COOPER and CAMPBELL have analysed samples of the snuff to which the Bantus are addicted. One of the compounds identified, 3 : 4-benzyprene, is strongly carcinogenic and another, 1 : 12-benzperylene, is very weakly so (p. 528).

THOMLINSON and GRAY have made a quantitative examination of many histological sections of lung cancers, mainly poorly differentiated squamous carcinomata, to determine the distance from the nearest vascular stroma at which necrosis develops in solid cords of tumour cells. They discuss the implications of their findings for the radiotherapy of such growths in the light of the known variation of radiosensitivity of tumour cells with oxygen tension (p. 539).

MORSON describes a series of gastric polyps, some of which were composed of mucosa of intestinal type and had apparently arisen in areas of intestinal metaplasia. The relationship between gastric polyps, pernicious anaemia and carcinoma of the stomach is briefly discussed (p. 550).

PRICE analyses a series of osteogenic sarcomata for the factors of age and sex distribution and incidence. He compares and contrasts his findings with those in other series of the same tumours, and also with groups of cases of primary chondrosarcoma, lymphosarcoma and fibrosarcoma (p. 558).

CALDWELL presents three examples of the association of a dermatomyositic symptom-complex with malignant disease (p. 575).

AHLSTRÖM and ISING describe the adaptation of the Ehrlich mouse ascites cancer to growth in the peritoneal cavity of the hamster. The "hamster-adapted" line shows increased virulence in hamsters and decreased virulence in mice as compared with the line of origin (p. 582).

ISING finds that the adaptation of the Ehrlich mouse ascites tumour to growth in the hamster is associated with a reduction in chromosome number and that this lower number is maintained after transfer back to mice (p. 592).

COHEN and COHEN describe the characteristics of a tumour resulting from a sudden spontaneous mutation which occurred in one first passage C3H mouse mammary carcinoma homoplast. The mutant tumour, though morphologically similar to other mammary tumours of the strain, grows faster and is more radiosensitive (p. 600).

KENNAWAY, TIPLER and URQUHART report failure to produce tumours in guinea-pigs with three agents carcinogenic in other species. They discuss this resistance in relation to certain unusual features of the guinea-pigs' metabolism of ascorbic acid, a substance which they cannot synthesise (p. 606).

URQUHART finds that the administration of 2-acetamidofluorene to the guinea-pig with the food has no carcinogenic action such as is seen in the rat, though both species produce the 7-hydroxy derivative (p. 611).

PULLINGER records observations on the incidence of spontaneous benign and malignant mammary tumours in a strain of mice without the milk factor; and relates their prevalance to the age and parity of the hosts (p. 613).

PULLINGER finds that the incidence of benign mammary growths in old female mice, which is decreased fifteen-fold by ovariectomy after breeding, is restored to about its original level when the animals are given substitution treatment with oestrone. The incidence of malignant tumours is not increased by oestrone in the doses used (p. 620).

ORR describes the epilation, necrosis and subsequent regeneration which follow a single application of 9 : 10-dimethyl-1 : 2-benzanthracene to areas of the skin of mice and discusses the significance of the process of repair in relation to the results of previously reported grafting experiments (p. 623).

LENNOX describes the skin tumours which followed painting the lumbar skin of rats and mice with 2-anthramine (p. 631).

JONES records the development of pure interstitial cell growths in intrasplenic testicular homotransplants in castrated, but not in normal, rats. None of the tumours showed evidence of malignancy (p. 640).

BALDWIN has treated rats bearing grafts of tumours, originally induced within the same inbred strain of animals, with ethanol extract concentrates of the homologous tumours. The tumour extracts cause considerable tissue destruction when injected into growing tumours, and a few tumours regress completely and their hosts remain immune to further implants of the same tumour. The bearing of these findings on the development of tumour immunity is discussed (p. 646).

BALDWIN finds considerable variation in the immune response evoked in rats following regression of transplanted tumours originally induced with methylcholanthrene (p. 652).

ABRIGNANI and MUTOLO present results suggesting that the lowering of the liver catalase activity in rats fed with 4-dimethylaminoazobenzene is related to an anticatalase substance brought about by the metabolic changes which occur in the liver during the neoplastic induction, rather than to a direct action of the azo dye upon the catalase (p. 658).

CAUSEY and HOFFMAN describe the electron microscope appearances of rat dorsal root ganglion cells. They believe that mitochondria may be derived from vesicles formed from the nuclear membrane (p. 666).